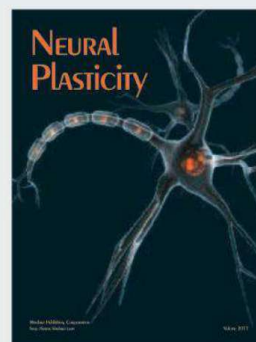
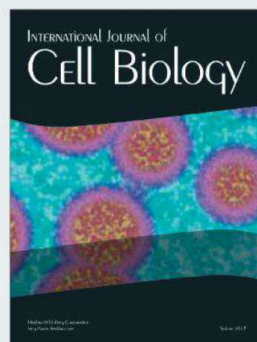
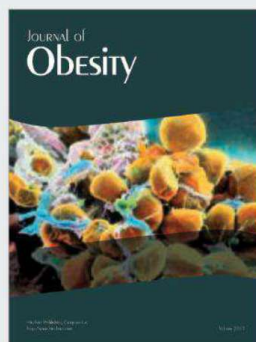
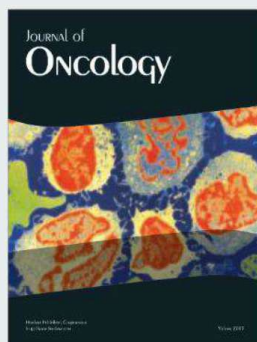
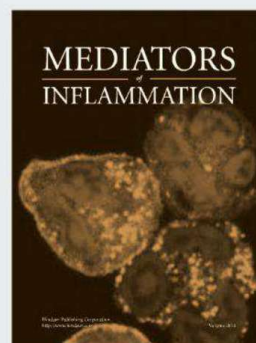
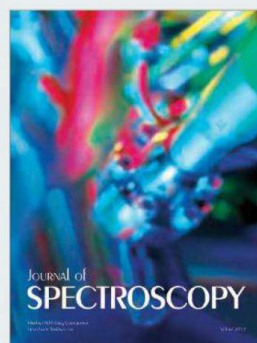
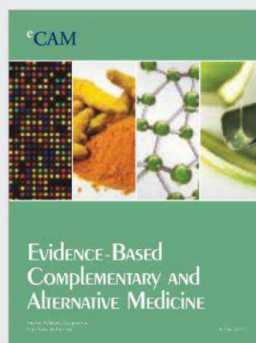
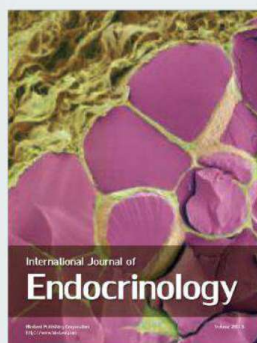
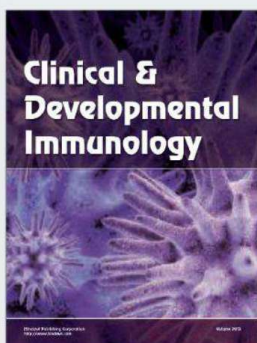
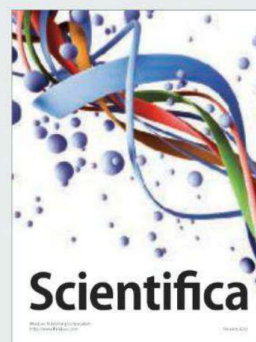
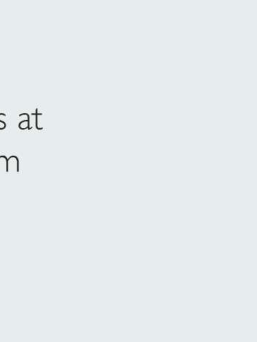
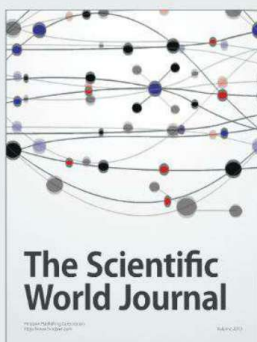
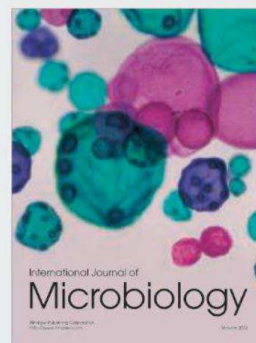
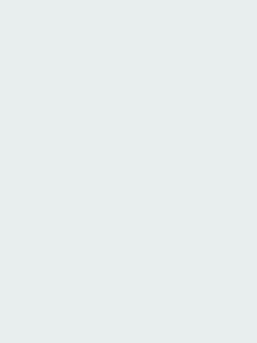
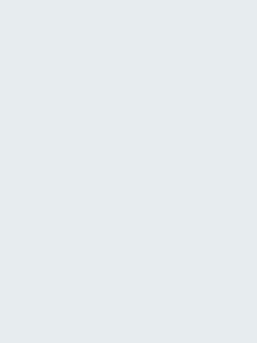
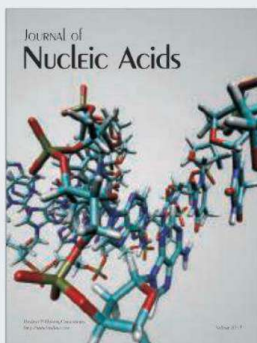
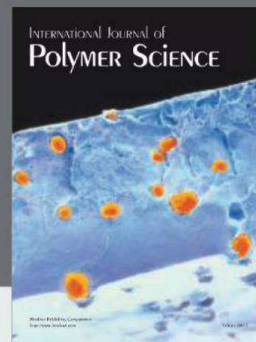
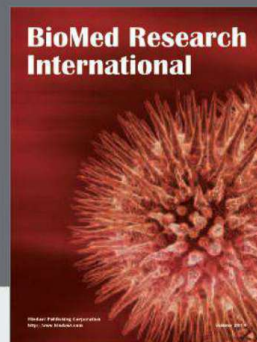
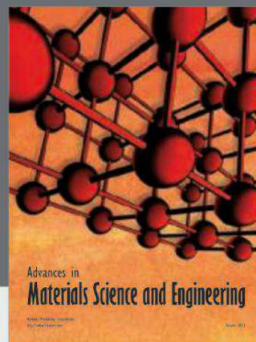
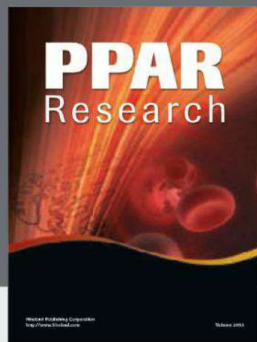
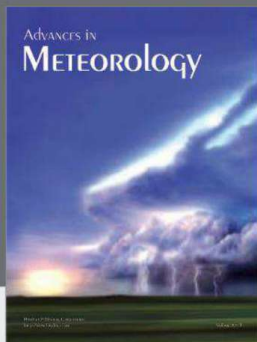


Science

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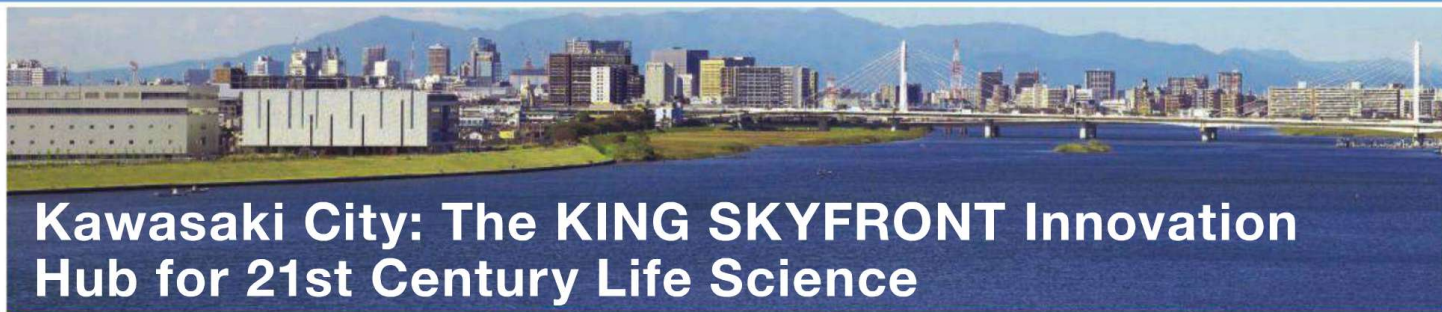
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Award Categories

- Photography
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Kawasaki City: The KING SKYFRONT Innovation Hub for 21st Century Life Science

Adjacent to Tokyo International (Haneda) Airport lies a unique 40-hectare science and technology innovation hub, the Kawasaki INnovation Gateway (KING) SKYFRONT. The mission of this futuristic facility is to find actionable solutions to issues in regenerative medicine, cancer treatment, and the prevention of 'lifestyle' diseases such as obesity. Importantly, the Japanese government has designated the KING SKYFRONT to be a special zone for international competitiveness development, bestowing it special privileges including reduced corporate taxes and government-backed financial support for projects launched there.

"KING SKYFRONT is the first important step in establishing the Tonomachi area of Kawasaki City as Asia's Silicon Valley," says Nobuhide Kobayashi, executive director of the Coastal Area International Strategy Office, Kawasaki City. "We are confident that the easy access provided by Haneda Airport, in conjunction with the excellent research and development infrastructure, will form the basis for unprecedented business opportunities growing out of innovative science, medicine, and technology." Kobayashi has reason to be confident. Kawasaki City, with its 1.4 million inhabitants, is already home to many blue chip companies including Aji-

nomoto, Toshiba, Fujitsu, and NEC as well as other global brands that have played a central role in the growth of Japan's economy over the last century.

The KING SKYFRONT is at the heart of the Keihin Industrial Region, encompassing areas of Tokyo and Kanagawa Prefecture, and Haneda Airport. This location offers unprecedented land, sea, and air access to cities within Japan, and the international airport connects the area to Asia, North America, and Europe. "Life science is one of the strategic areas for KING SKYFRONT," says Kobayashi. "We envisage that visiting scientists, medical doctors, and business people will be able to arrive at Haneda in the morning, go directly from the airport to KING SKYFRONT, participate in meetings, and return to their country on an evening flight."

In addition to the excellent technological infrastructure and access, KING SKYFRONT is located within commuting distance of international schools and medical clinics run by English-speaking clinicians—important factors to consider for creating long-term collaborations with overseas partners.



Kazunori Kataoka

Launch of the innovation Center Of Nanomedicine

The innovation Center Of Nanomedicine (iCON) is set to be the flagship of the KING SKYFRONT development. With government startup funding of approximately ¥3.5 billion (US\$34.7 million), construction on the project is due to start in 2013, with a formal launch in 2014. Re-

search at the center—carried out by groups from academia, industry, and government—will address such diverse issues as the medico-economic impact of aging populations, curbing the increasing cost of medical care, improving point of care treatment, and establishing new medical markets in developing countries.

Translation of research ideas to the marketplace and fabrication/manufacture are two of the main strategic pillars of the center, emphasizes Professor Kazunori Kataoka of the University of Tokyo, who will be director of research in nanomedicine at the center. "The KING SKYFRONT will offer an open-innovation approach to meet our targets," says Kataoka. "The proximity of Haneda Airport, the availability of clean rooms and related infrastructure for nanofabrication, industrial participation, and professionals to advise on the commercial aspects of research are essential ingredients for taking creative ideas from the laboratory to the marketplace. KING SKYFRONT can be viewed as a highly condensed version of Silicon Valley."

Kataoka is no stranger to big ideas and translational research, having launched the company NanoCarrier in 1996 to commercialize nanosized micelles for targeted drug delivery for the treatment of cancer and other

intractable diseases. Notably, NanoCarrier is listed on the *Mothers* (market of the high-growth and emerging stocks) section of the Tokyo Stock Exchange and valued at ¥100 billion (US\$985 million).

Underscoring Kataoka's internationally recognized contributions to innovative research in nanobiotechnology, he recently received support from Japan's prestigious national Funding Program for World-Leading Innovative Research and Development on Science and Technology (FIRST) program. With funding of ¥3.6 billion (US\$35.7 million) over five years, Kataoka's team will address the following topics: (1) Treatment of cancer stem cells using targeted drug delivery systems in which nanometer-sized drug carriers are able to selectively bind to cancer cells; (2) Treatment of neurological diseases such as Alzheimer's disease using functionalized micelles capable of passing through the blood-brain barrier; (3) Development of nanovaccines that can be stored for long periods of time at room temperature; (4) Fusion of drug delivery platforms with medical devices for minimally invasive "chemical surgery"—such as ultrasonic waves that activate drugs at specific places in the body—to reduce the length of hospital stays for patients; and (5) Development of point-of-care diagnostic devices, such as the use of portable nanofabricated systems to detect microRNAs and specific enzymes.

Kataoka emphasizes the importance of what he calls 'smart healthcare.' "Reducing the time a patient stays in hospital would be a tremendous contribution to society. I am confident that research at iCON can achieve this." As an example of how nanomedicine may eventually be used by millions of people, Kataoka describes the development of the advanced technology necessary to produce hybrid cars. "These cars require a lot of expensive technology. But mass production has enabled cost reductions to within the price range of millions of people worldwide. I predict a similar scenario for nanomedicine."



Artist's Impression of the innovation Center Of Nanomedicine (iCON)



Hiromichi Kimura



Tomohiro Anzai

Participants in the innovation Center Of Nanomedicine include:

Academia and National Institutes

The University of Tokyo
Tokyo Institute of Technology
Tokyo Women's Medical University
National Cancer Center (Tokyo)
Central Institute for Experimental Animals
Keio University (Tokyo)

Stanford University (USA)
Karolinska Institutet
(Sweden)

Industry

Fujifilm Corporation
Nikon Corporation
NanoCarrier Co., Ltd.

“We will not only have people from different academic backgrounds but there will also be people from industry as well as government administrators.” —Hiromichi Kimura

From Lab to Market

Technology transfer at iCON will be managed by two professors from the University of Tokyo: Hiromichi Kimura, in charge of Pharmaco-Business Innovation at the Graduate School of Pharmaceutical Sciences, and Tomohiro Anzai, a member of the Translational Research Initiative.

Kimura's academic background is in biochemistry. After graduating from the University of Tokyo, he joined a major life sciences-based company in Japan before moving to the U.S. to complete a Master of Business Administration and Ph.D. at Stanford University. “I now have my own consultancy company as well as the post at the University of Tokyo,” says Kimura. Anzai is also a graduate of the University of Tokyo where he trained as a molecular biologist. He spent some time at a consultancy company, because he “was interested in the commercialization of research ideas,” before joining KING SKYFRONT. Anzai also works with the University of Tokyo Hospital on technology transfer.

Kimura and Anzai stress that the interdisciplinary nature of the projects at KING SKYFRONT and iCON will require good communication skills between project members. “We will not only have people from different academic backgrounds but there will also be people from industry as well as government administrators,” says Kimura. “Our biggest challenge will be to formulate ways to ensure that physicists, medical doctors, and chemists can communicate with investment bankers, lawyers, and patent attorneys. They may speak different languages, but they have the same goal of making iCON a success.”

Laying the Foundations for a Global Innovation Hub

The Central Institute for Experimental Animals (CIEA)—internationally recognized for research on the use of induced pluripotent stem cells for the treatment of spinal cord injuries and Alzheimer's disease—was the first major research institute to move to KING SKYFRONT, transferring its facilities in July 2011.

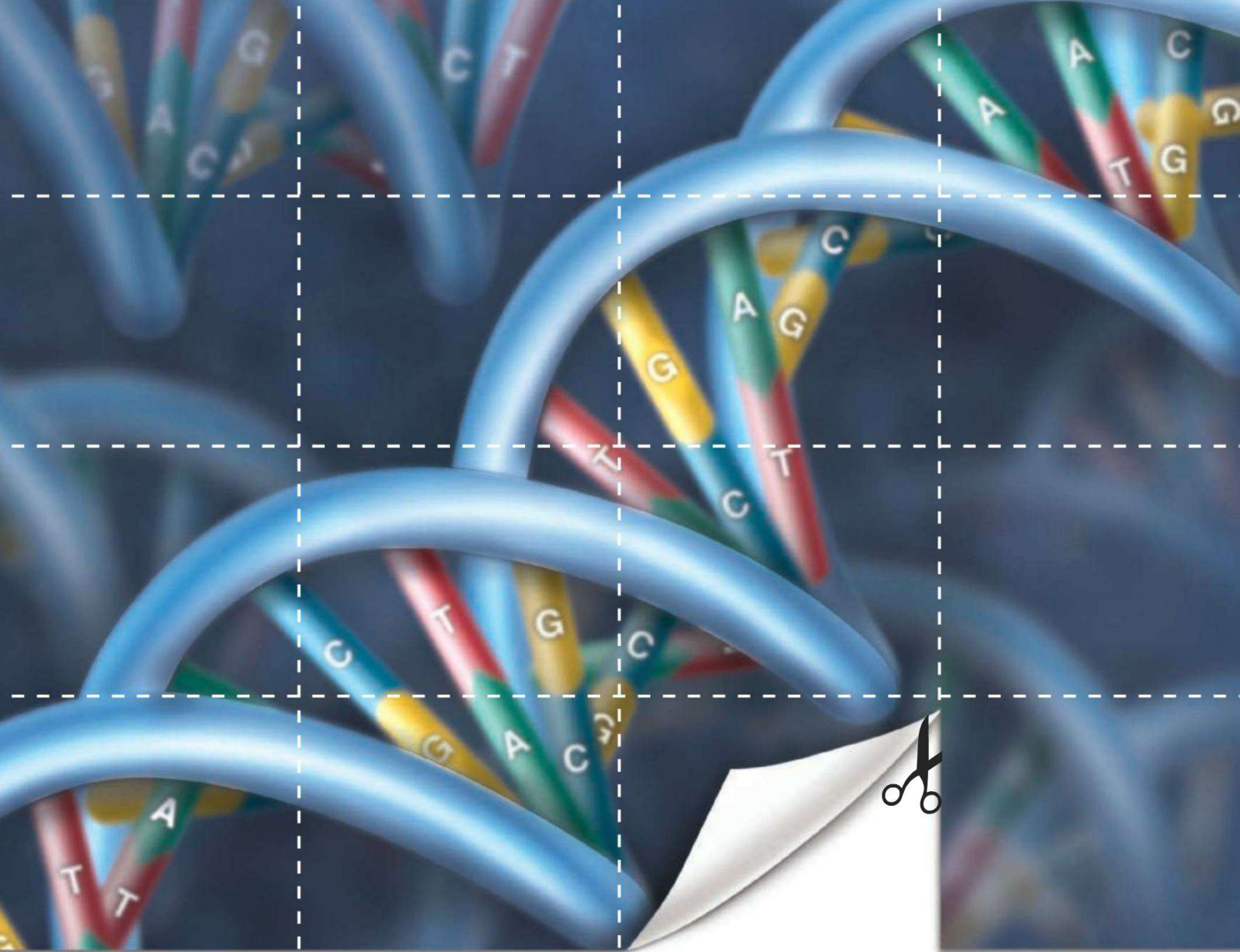
March 2013 saw the opening of the Life Science and Environment Research Center (LiSE) to promote collaborative research in the life sciences, and consisting of a multipurpose facility housing laboratories from both Kawasaki City and the private sector.

In 2016, the Japanese National Institute of Health Sciences (NIHS) will move from Tokyo to the KING SKYFRONT. The NIHS plays a central role in regulatory science in Japan and at KING SKYFRONT it will establish global standards for new drugs and medical technology developed by researchers there.

KING SKYFRONT has attracted attention from overseas companies including Johnson and Johnson, which will establish an R&D and training center onsite.

“The world's life sciences community will continue to ‘organically’ lay the foundations for a global innovation hub for research,” says Kobayashi. “It's an exciting time at Kawasaki City as KING SKYFRONT evolves into a global base for research in the 21st century.”





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COVER

Clouds pass over Kenya's Lake Magadi, where geologists are drilling a core to gather detailed climate data from the ancient lake bed. Data on past climates are vital for researchers seeking to understand how anthropogenic climate change will affect Earth's ecosystems and species, including its effects on infectious diseases and food security. See the special section beginning on page 472.

Photo: © Martin Harvey/Alamy

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Want to win a rather special prize in Stockholm, Sweden this December?



Henrik Torgs / image bank, Sweden

**Winner's essay published in the journal *Science*
\$25,000 dollars grand prize
Awards held in Stockholm in December**



This December a rather special prize will be awarded in Stockholm, Sweden. The journal *Science* and SciLifeLab have come together to recognize and celebrate excellence in PhD research. The *Science* and SciLifeLab Prize has been established to support young scientists at the start of their career.

“Science has never been more exciting and, as leaders in science, we need to support and encourage young researchers today and tomorrow. This prize is a way of doing just that.”

Professor Mathias Uhlén, Director SciLifeLab

The grand prize winner of this major global award will have their essay published in the journal *Science* and receive \$25,000. Three runners up will receive a combined total of an additional \$10,000 in prize money.

The prizes will be presented in Stockholm, Sweden in the middle of December 2013.

To enter

You must be a recent Ph.D. graduate (awarded between January 1, 2011 and December 31, 2012).

Submissions must be in the form of a 1000 word essay, in English, on your thesis, highlighting the significance of its contribution and overall implications in the field. The four submission areas for this prize are:

- (1) Genomics / Proteomics / Systems Biology
- (2) Developmental Biology
- (3) Molecular and Cellular Biology
- (4) Environmental Life Science.

The deadline for submissions is August 15, 2013. The overall winning essay will be published in *Science*.

For further details and to enter, please go to: www.sciencemag.org/scilifelabprize

*For over 130 years the journal *Science* has been the world's leading journal of original scientific research, global news and commentary.*

SciLifeLab is a collaboration between four universities in Stockholm and Uppsala, Sweden, and is a pioneering center for large-scale biosciences with a focus on health and environmental research.

With the kind support of the Knut and Alice Wallenberg Foundation.

*Knut och Alice
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SciLifeLab

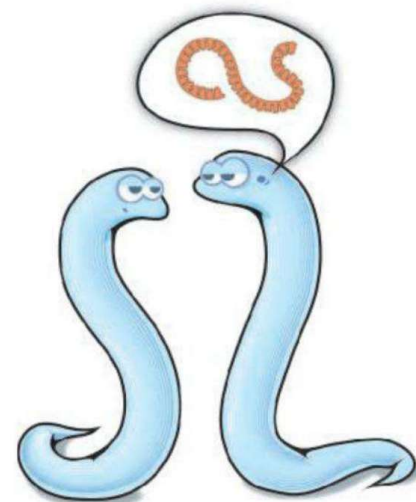
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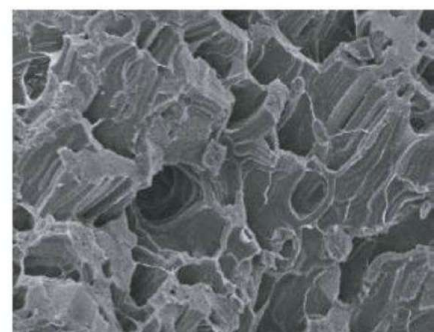
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Doppler Effect with a Twist

The Doppler shift is a familiar and well-understood effect in acoustics. Radar guns use the same effect to determine the speed of moving vehicles. Applied to a rotating object side-on, however, a linear Doppler effect would register no movement. Using twisted light, whereby photons are imprinted with a given amount of optical angular momentum, Lavery *et al.* (p. 537; see the Perspective by Marrucci) detected rotation with an analogous angular Doppler shift, which may be useful for remote sensing and observational astronomy.

Examining Y

The evolution of human populations has long been studied with unique sequences from the nonrecombining, male-specific Y chromosome (see the Perspective by Cann). Poznik *et al.* (p. 562) examined 9.9 Mb of the Y chromosome from 69 men from nine globally divergent populations—identifying population and individual specific sequence variants that elucidate the evolution of the Y chromosome. Sequencing of maternally inherited mitochondrial DNA allowed comparison between the relative rates of evolution, which suggested that the coalescence, or origin, of the human Y chromosome and mitochondria both occurred approximately 120 thousand years ago. Francalacci *et al.* (p. 565) investigated the sequence divergence of 1204 Y chromosomes that were sampled within the isolated and genetically informative Sardinian population. The sequence analyses, along with

archaeological records, were used to calibrate and increase the resolution of the human phylogenetic tree.

The Only Flame in Town

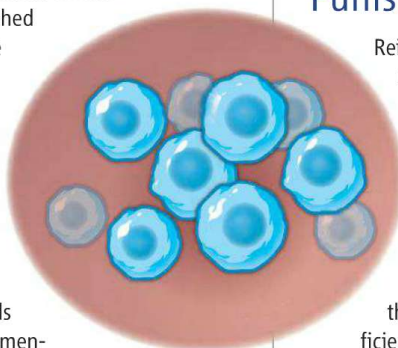
Unusual for mammals, humans are notably socially monogamous, with pair bonding sometimes lasting decades. Why? Lukas and Clutton-Brock (p. 526; see the Perspective by Kappeler) examined data from over 2500 mammalian species across 26 orders containing 60 evolutionary transitions to monogamy. In every case, the ancestral condition was one where females were solitary and where male infanticide was unusual. Monogamy appears to arise not as a response to a need for paternal care, but largely as a mate-guarding strategy.

A Complicated Scaffold, Simply

Materials with tailored pore structures can be useful as catalysis supports and for lightweight materials. When preparing medical scaffolds, restrictive preparation conditions have to be met, which can prohibit multistep preparation procedures. Sai *et al.* (p. 530) describe a method for making porous polymers containing both relatively large (several microns) interconnecting pores and a second population of ~ tens of nanometer pores. The process exploits spinodal decomposition of a block copolymer blended with small-molecule additives and requires a simple washing step with water, methanol, or ethanol.

Protecting the Guts

Regulatory T cells (T_{reg}) in the gut are important sentinels in maintaining the peace between our gut and its trillions of resident bacteria and have been shown to be regulated by specific strains of bacteria in mouse models. Smith *et al.* (p. 569, published online 4 July; see the Perspective by Boll-rath and Powrie) asked whether metabolite(s) generated by resident bacterial species may regulate T_{reg} s in the gut. Indeed, short-chain fatty acids (SCFAs), bacterial fermentation products of dietary fibers produced by a range of bacteria, restored colonic T_{reg} numbers in mice devoid of a gut microbiota



and increased T_{reg} numbers in colonized mice. The effects of SCFAs on T_{reg} s were mediated through GPCR43, a receptor for SCFAs, which is expressed on colonic T_{reg} s. Mice fed SCFAs were protected against experimentally induced colitis in a manner that was dependent on GPCR43-expressing T_{reg} s.

Isothermal Water Splitting

Solar concentrators can create extremely high temperatures that can drive chemical reactions, including the thermal splitting of water to provide hydrogen. A metal oxide catalyst is needed that is usually cycled between hotter conditions where it is reduced and cooler conditions where it is reoxidized by water. This cycling can limit catalyst lifetime, which can be costly. Muhich *et al.* (p. 540; see the Perspective by Roeb and Sattler) developed an approach that allowed the redox cycle to be driven isothermally, using pressure swings.

Hot and Bothered

One of the most uncertain aspects of energy production at geothermal fields is the potential for the induction of earthquakes from extraction of hot fluids and injection of wastewater back into the subsurface. Brodsky and Lajoie (p. 543, published online 11 July) compared the historical rates of seismicity—after correcting for the occurrence of aftershocks—to operational parameters at the Salton Sea Geothermal Field in southern California. The net volume of fluid extracted and injected was correlated with recent seismicity. However, since the 1980s, when the first plant came online, the rate of earthquakes induced with additional increments of volume injected has decreased.

Coding Reward Versus Punishment

Reinforcement learning is driven by reward prediction error, and a very influential theory has proposed that dopamine neurons provide this signal to teach value to the brain. Although this is called a reward prediction error, it has been assumed to also represent aversiveness. Thus, it was thought that the dopamine signal could be sufficient for learning total value. Fiorillo (p. 546) found that dopamine alone was not sufficient to encode value, implying that there must be an analogous signal for aversiveness.



Marcia McNutt is Editor-in-Chief of *Science*.

Climate Change Impacts

ANTICIPATING THE FUTURE UNDER THE INFLUENCE OF CLIMATE CHANGE IS ONE OF THE MOST important challenges of our time, and the topic of the special section in this issue of *Science* (see p. 472). The natural systems that provide oxygen, clean water, food, storm and erosion protection, natural products, and the potential for future resources, such as new genetic stocks for cultivation, must be protected, not just because it is part of good stewardship but also so that they can take care of us. But even the first step of modeling the effects of greenhouse gas sources and sinks on future temperatures requires input from atmospheric scientists, oceanographers, ecologists, economists, policy analysts, and others. The problem is even more difficult because the very factors that influence temperature changes, such as ocean circulation and terrestrial ecosystem responses, will themselves be altered as the climate changes. With so many potential climate-sensitive factors to consider, scientists need ways to narrow down the range of possible environmental outcomes so that they know what specific problems to tackle.

Researchers have turned to the geologic record to obtain ground truth about patterns of change for use in climate models. Information from prior epochs reveals evidence for conditions on Earth that might be analogs to a future world with more CO₂. Projections based on such previous evidence are still uncertain, because there is no perfect analog to current events in previous geologic epochs; however, even the most optimistic predictions are dire. For example, environmental changes brought on by climate changes will be too rapid for many species to adapt to, leading to widespread extinctions. Even species that might tolerate the new environment could nevertheless decline as the ecosystems on which they depend collapse. The oceans will become more stratified and less productive. If such ecosystem problems come to pass, the changes will affect humans in profound ways. The loss in ocean productivity will be detrimental for the 20% of the population that depends on the seas for nutrition. Crops will fail more regularly, especially on land at lower latitudes where food is in shortest supply. This unfavorable environmental state could last for many thousands of years as geologic processes slowly respond to the imbalances created by the release of the fossil carbon reservoir. The time scale for biodiversity to be restored, with all the benefits that it brings, will be even longer.

Unfortunately, I view these predicted outcomes as overly optimistic. We are not just experiencing increases in greenhouse gas emissions but also eutrophication, pollution of the air and water, massive land conversion, and many other insults, all of which will have interacting and accumulating effects. The real problem we need to solve in order to truly understand how Earth's environment may change is that of cumulative impacts. Although the Paleocene-Eocene Thermal Maximum (about 55 million years ago) is the time period considered to be a reasonable analog to a higher-CO₂ future, the planet was not experiencing these other stressors and climate change simultaneously. So terrestrial species that survive a climate impact alone may face extinction if reduced to a fraction of their natural range through deforestation and habitat fragmentation. Marine species that are mildly susceptible to ocean acidification may not be able to tolerate this condition plus low oxygen levels.

Sometimes the science of cumulative impacts is straightforward—for example, connecting habitats to provide migration corridors in response to sea-level rise brought on by climate change. But even “clear-cut” cases require extra work, more partnerships, and more time to address. Tackling problems of cumulative dimensions is a priority if we are to find viable solutions to the real environmental crises of the coming decades. There is a need for all scientists to rise to this challenge.

— Marcia McNutt

10.1126/science.1243256

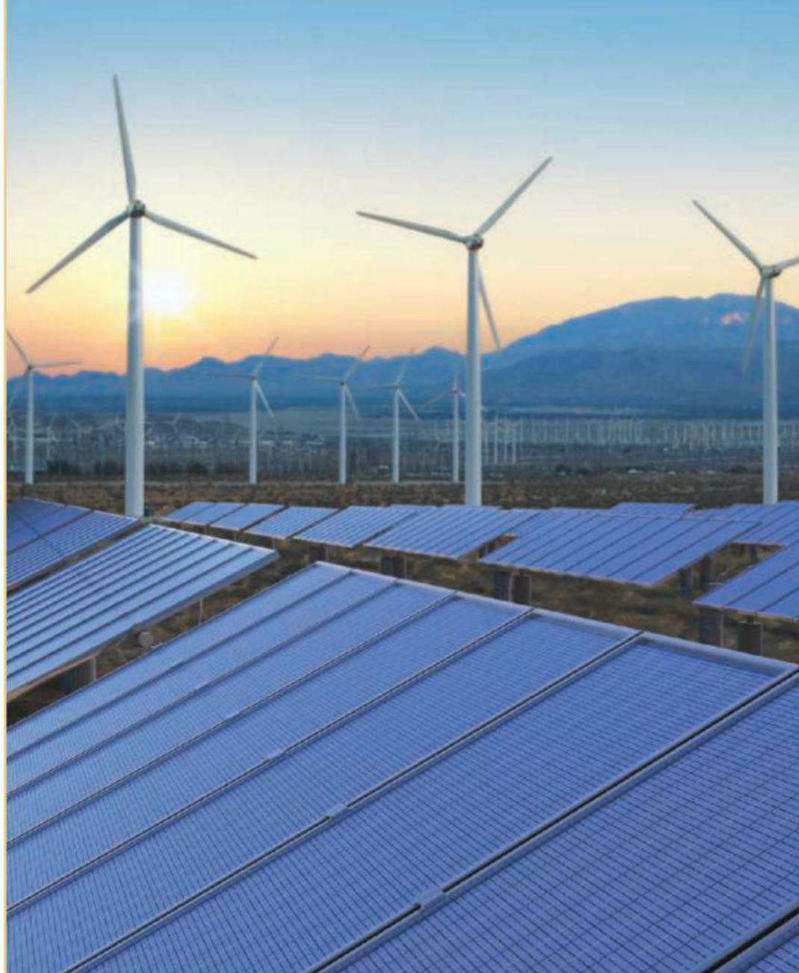


ENVIRONMENTAL SCIENCE

Renewable Benefits

Few would doubt the proposition that wind and solar power provide health and environmental benefits by reducing emissions of carbon dioxide and other air pollutants, although few have considered in depth how the advantages of those renewable energy technologies might differ regionally. The variability of the advantages of renewable power is largely a function of the type of power generation that it displaces. Siler-Evans *et al.* look at how existing power generation facilities are distributed across the United States and analyze the impacts of replacing those facilities with wind or solar installations. They find uneven but significant—and in some regions, very large—social and environmental benefits resulting from the adoption of those technologies. They also discuss the value of the Production Tax Credit Subsidy for wind energy generation, how its effectiveness could be improved by regional differentiation of the policy, and how the large-scale adoption of wind or solar energy production might affect their more site-specific analysis. — HJS

Proc. Natl. Acad. Sci. U.S.A. **110**, 11768 (2013).



EVOLUTION

Open to Change

Changes in the regulation of gene expression can result in changes in organismal phenotypes. Nakagawa *et al.* have used phylogenetic analysis and biochemical measurement to study DNA binding specificity changes in the forkhead box (Fox) family of transcription factors. This family is present in a wide range of species and is one of the largest classes of transcription factors in humans. They used phylogenetic inference methods to examine the relationship of Fox domain sequences spanning 623 genes from 65 species, and they characterized binding specificity in vitro for 21 Fox proteins and combined this with published binding data for 9 proteins. They found that changes in specificity from canonical forkhead primary (FkhP) and forkhead secondary (FkhS) motifs to alternative DNA sequences have occurred separately in three different Fox subfamily lineages. In fungal Fox3 proteins, two specificities have arisen, FHL-3 and FVH, but only FVH binding involves the mutation of residues involved in DNA recognition. In the metazoan FoxM subfamily, proteins retain specificity for FkhP and FkhS but are also able to bind an FHL motif, whereas in the holozoan FoxN subfamily, some proteins exhibit a similar bispecificity, yet others have lost the ability to bind the

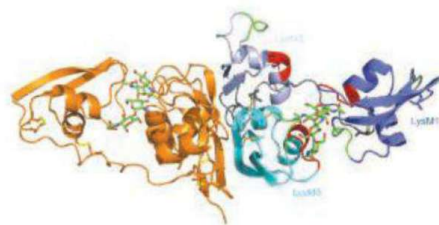
canonical sites and only bind FHL-N. Fox domains that display bispecific binding are probably able to switch between two conformations with distinct DNA binding specificities. Such bispecificity may be central in the evolution of transcriptional regulatory networks. — VV

Proc. Natl. Acad. Sci. U.S.A. **110**, 10.1073/pnas.1310430110 (2013).

MICROBIOLOGY

Hide and Seek

Plants and their fungal pathogens are at war. Plant surface receptors, which contain lysin motifs (LysMs), sense fungal chitin oligomers, which are basic components of fungal cell walls, and



thereby trigger immune defenses against the fungus. The fungi, in turn, have evolved molecular countermeasures. Sanchez-Vallet *et al.* report

structural studies of a fungal effector protein, Ecp6, which is secreted by the leaf mold *Cladosporium fulvum* and provides a means for the pathogen to hide from the host. Ecp6 also contains LysM, but unlike the plant's receptors, the fungal motifs dimerize in the presence of its own chitin to form a deeply buried groove that binds chitin with high affinity and keeps it out of sight of the plant's immune responses. LysM seems to be ubiquitous among fungi and may represent a common mechanism by which such pathogens can evade host defenses. It is interesting that two evolutionarily distant organisms have converged on the ability to recognize the same molecule via the same motif that resides in divergent proteins and with antagonistic effect. — CA

eLife **2**, e00790 (2013).

PLANT SCIENCE

Location and Timing

In plants, the circadian network coordinates physiological processes with the daily rhythms of light and dark. The protein GIGANTEA (GI) is found in both nuclear and cytoplasmic compartments of the plant cell and regulates different partners in the two locations. Using *Arabidopsis*, Kim *et al.* constructed plants with disrupted subcellular localizations of GI. Their modeling analyses of the

mutant plants show that GI in the nucleus acts as a positive regulator of the core oscillator gene *LATE ELONGATED HYPOCOTYL (LHY)*, whereas GI in the cytoplasm acts as a negative regulator. This regulatory cycle supports a peak of *LHY* in the morning and a shift to the control of flowering genes by GI in the evening. The spatial segregation and opposition of function make the circadian system more robust in the face of stochastic noise; the models suggest that using one component in opposing ways generates a more stable system than does using two separate components. — PJH

Dev. Cell **26**, 1016/j.devcell.2013.06.006 (2013).

MICROBIOLOGY

The Social Life of Small Spaces

Bacteria have traditionally been considered as swarms of independent cells, but the notion of bacteria as members of populous and integrated communities is becoming more prevalent. For instance, the existence of diffusible signals that contribute to quorum sensing among groups of bacteria to coordinate their responses to the environment is now well established. Remis *et al.* have looked at how *Myxococcus xanthus*, a social bacterium present in soil, can communicate via direct contact mechanisms. Artifact-free preservation and electron microscopic 3D imaging of cell structures revealed the fine structure of individual bacteria and their membrane appendages, as well as cellular 3D biofilm organization. Lipid-based vesicle chains and membrane tubes were observed that increased in number in biofilms and could facilitate direct communication between linked bacteria. The idea that bacteria in a biofilm can intimately connect at the level of the periplasm suggests that biofilm communities can be viewed as a "superorganism" that transfers membrane proteins and other molecules between cells in order to coordinate group behavior. — SMH

Environ. Microbiol. **15**, 10.1111/1462-2920.12187 (2013).

PHYSICS

The Higher Andreev State

In superconductors, materials capable of perfect conduction of electricity, the associated supercurrent is carried by pairs of electrons known as

Cooper pairs. If a superconductor is placed in contact with a nonsuperconducting material, a single electron impinging on the interface from the nonsuperconducting side is reflected as a hole and forms a Cooper pair on the superconductor side—a process known as Andreev reflection. For a nonsuperconducting material sandwiched between two superconductors (as is the case in devices such as tunnel Josephson junctions), the reflections on the two interfaces will cause the formation of standing waves—Andreev bound states (ABSs)—which are responsible for carrying the supercurrent across the weak link. The ABSs have a discrete spectrum, but usually only the lowest energy state is accessed in experiments. Bretheau *et al.* directly detected an excited ABS in a less common form of a Josephson junction, where the weak link is an atomic contact. Their apparatus consisted of an atomic contact junction in parallel with a tunnel junction, and with another tunnel junction acting as a source and detector of microwaves. By measuring the current and voltage dependence of the latter, and by varying the phase difference across the atomic contact, they were able to precisely map out the energy needed to excite the ground ABS. It is expected that the existence of two ABSs can be used as a basis for a quantum bit or as a stage for exploring fundamental mesoscopic phenomena. — JS

Nature **499**, 312 (2013).

PHYSICS

Solid-State Optical Storage

Photons are ideal carriers of information. They are fast and coherent, and because they don't interact much with each other they can have very long lifetimes. However, stopping, processing, and manipulating the photons requires them to be stored in a memory, where doing so can damage their fragile quantum properties, which leads to a loss of coherence. Although photons have been coherently stored in and retrieved from ensembles of atom gases, both warm and cold atom clouds, for practical applications a solid-state memory would be desired. Heinze *et al.* have developed a method to enhance the storage time of photons in a rare-earth-ion-doped crystal. They use a series of laser pulses to initialize the crystal for photon storage and then load it with their "information photons." By iteratively optimizing the experimental parameters, they show that they can store light pulses and even entire images in the crystal and then retrieve them up to a minute later with their coherent properties intact. The results present an important step toward building a viable quantum network. — ISO

Phys. Rev. Lett. **111**, 033601(2013).

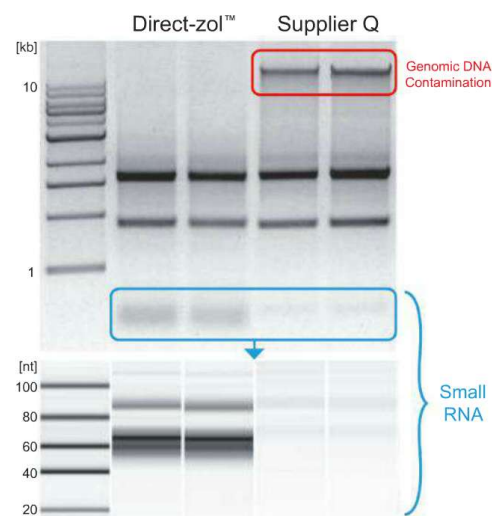
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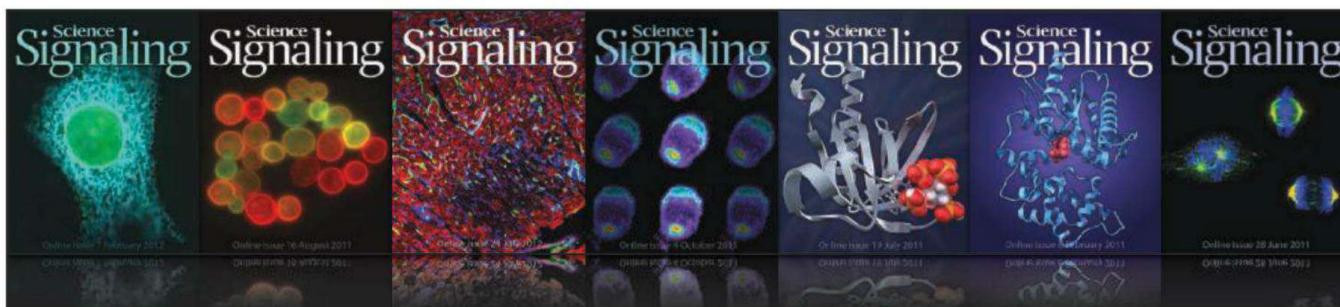
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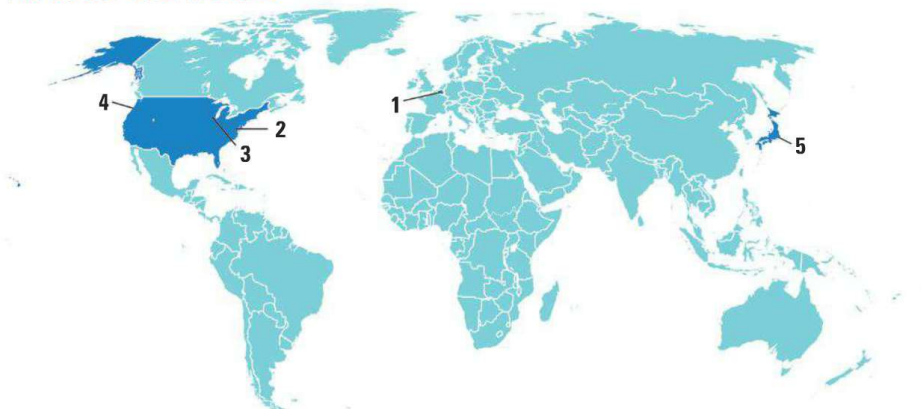
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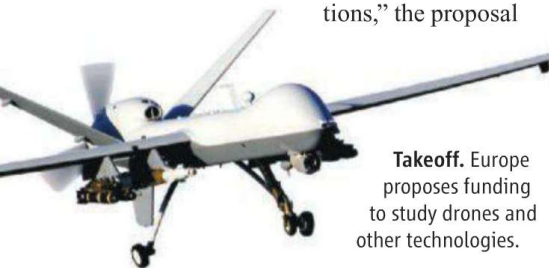


Brussels 1

E.C. Lays Groundwork For Defense Research

In an attempt to bolster Europe's ability to counter global security threats, the European Commission has proposed that the European Union consider funding military research. Observers say that the proposal could encourage cooperation in an area that has largely remained the turf of individual countries.

The commission is not suggesting using the region's primary vehicle for cooperative research, Horizon 2020. That program will keep an "exclusive focus on civil applications," the proposal



Takeoff. Europe proposes funding to study drones and other technologies.

says. But the commission wants to see if some technologies funded by Horizon 2020 could also be used in defense applications.

Commission President José Manuel Barroso presented the plans on 24 July, along with two European commissioners. "Twenty years ago it wouldn't have been natural for the commission to talk about defense. [But] times have changed," Barroso told reporters. Spending on military research in E.U. member states dropped by 14% from 2005 to 2010, and the unspoken fear is that Europe may be falling behind in developing new technologies such as drones and systems for detecting new biological, chemical, and nuclear weapons. The proposal will be discussed at the European Council meeting in December. <http://scim.ag/EUdefres>

Washington, D.C. 2

U.S. Alters R&D Accounting

The way the United States accounts for the value of research and development spending is getting a major makeover. On 31 July, the U.S. Bureau of Economic Analysis began factoring R&D expenditures by business and government—estimated at some \$400 billion last year—into its regular tally of the nation's gross domestic product (GDP), a key measure of economic health. In the past, GDP bookkeepers essentially counted R&D spending as an expense. Now, it is treated as an investment. Government analysts predict that the change will slightly boost annual GDP. If R&D had been treated as an investment between 2002 and 2007, for instance, average annual U.S. GDP growth would have been 0.12 points higher. Other studies estimate that, in 2007, R&D spending accounted for 3% of the roughly \$13 trillion U.S. GDP.

Batavia, Illinois 3

Giant Magnet Arrives at Fermilab

On 26 July, a 15-meter-wide superconducting magnet rolled through the gates of Fermi National Accelerator Laboratory (Fermilab) in Batavia, Illinois—the last step of a nail-biting 5000-kilometer journey that moved the delicate ring-shaped magnet from Brookhaven National Laboratory in Upton, New York, to its new home on the prairie.

The ring is part of the "Muon g-2" experiment, intended to precisely measure the magnetic moment of an elementary particle called the muon in hopes of detecting long-sought-after hints of new physics. An earlier experiment at Brookhaven produced a result that didn't agree with the standard model; now, the Fermilab team



Magnetic attraction. The Muon g-2 ring during its travel to Fermilab.

hopes to conclusively confirm or refute that result by repeating the experiment with an improved muon beam.

Fermilab scientists plan to start testing the magnet within the next few weeks, but won't know for sure whether the ring survived its journey unscathed until 1 year from now, when they try to cool its coils back down to superconducting temperatures. The team hopes to start taking data in 2016. <http://scim.ag/Fermimag>

Pacific Northwest 4

Killing One Species To Save Another

The U.S. Fish and Wildlife Service (USFWS) announced last week that it plans to dispatch hunters beginning this fall to shoot more than 3600 barred owls in California, Oregon, and Washington over the next 4 years. The cull is an attempt to prevent further population declines of the northern spotted owl, which is in danger of extinction.



Northern spotted owl

Barred owl

An emblem of the Endangered Species Act, northern spotted owls have seen their numbers sharply decline since 1990, when federal officials listed the birds as threatened. The listing halted logging in much of the Pacific Northwest's old growth forests, the spotted owls' preferred habitat. But barred

CREDITS (TOP TO BOTTOM): BROOKHAVEN NATIONAL LABORATORY; U.S. AIR FORCE PHOTO/STAFF SGT. BRIAN FERGUSON; GETTY IMAGES/FUSE; GETTY IMAGES/STOCKPHOTO

owls—which are larger, more aggressive, and less picky about what they eat—also inhabit these forests. Northern spotted owl numbers are declining by 2.9% each year, and “we can’t ignore the mounting evidence that competition from barred owls is a major factor,” says USFWS Director Daniel Ashe.

Once the barred owl hunt is complete, wildlife officials will monitor spotted owl populations and see whether they fare better than in regions taking no action against the barred owls. The hunting plan is expected to be finalized later this month.

Tokyo 5

More Hypertension Drug Probes

Another investigation has cast doubts on claims for a leading hypertension drug. In April 2007, results from the Jikei Heart Study, published in *The Lancet*, claimed that patients taking valsartan were at a lower risk of heart failure and stroke than those on alternatives. But on 30 July, an investigation at Tokyo’s Jikei University School of Medicine concluded that the *Lancet* paper “is fundamentally flawed” due to data manipulation and conflicts of interest.

Four other universities are conducting investigations; last month, investigators at Kyoto Prefectural University of Medicine found data manipulation in a valsartan paper based on a clinical trial at that school as well (*Science*, 19 July, p. 223). Valsartan is sold in Japan by Novartis Pharma, the Japanese arm of the Swiss pharmaceutical giant.

<http://scim.ag/hypdrug>

NEWSMAKERS

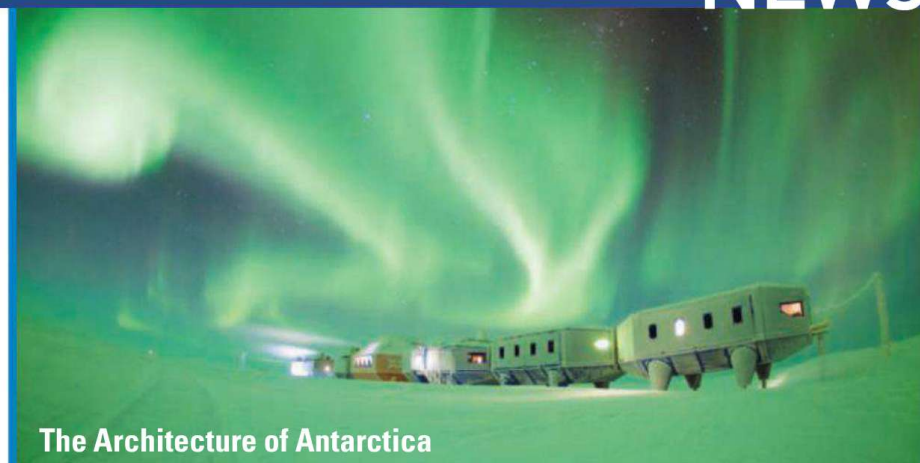
Head of European Food Agency Resigns

French food safety expert **Catherine Geslain-Lanéelle** unexpectedly stepped down as head of the European Food Safety Agency (EFSA) on 24 July to take up a new high-level job at the French Ministry of Agriculture, Food and Forestry. Her move came in the middle of her second term, slated to end in 2016. EFSA did not offer an explanation for Geslain-Lanéelle’s departure, but a source close to the agency says her new post was too good to pass up.

“She was deft at networking and her political judgment was good,” writes Joe



Geslain-Lanéelle



The Architecture of Antarctica

A research laboratory fit for the coldest, windiest, driest place on Earth must be sturdy, self-sufficient, and state-of-the-art—and represent its home country with pride.

Inspired by the construction 3 years ago of the United Kingdom’s fully relocatable and caterpillarlike Halley VI (pictured) on the Brunt Ice Shelf floating on the Weddell Sea, the British Council commissioned *Ice Lab*, a new exhibit of modern Antarctic labs that opened on 26 July at The Lighthouse in Glasgow (http://www.artscatalyst.org/projects/detail/ice_lab). The exhibit includes images of Belgium’s zero-emission Princess Elisabeth station, India’s shipping-container conglomerate Bharati Research Station, South Korea’s Death Star–like Jang Bogo, and a design by a Danish architect for a future station made entirely from ice.

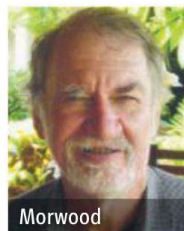
“Each station is really interesting in its own right,” says Vicky Richardson, director of architecture, design, and fashion at the council—and their construction doesn’t just reflect accelerating international interest in Antarctic research, she adds. “They’re almost a statement of national pride,” competing with one another in stylishness, size, and technical complexity.

Perry, chair of the agency’s panel on genetically modified organisms, in an e-mail. “I hope EFSA can get someone of her calibre as a replacement.”

EFSA was established in 2002, charged with providing independent scientific advice on the safety of food and feed in the European Union. Veterinary expert Bernhard Url, in charge of risk assessment at EFSA, will lead the organization until an interim executive director is appointed in October. EFSA’s management board will choose a new executive director from a shortlist to be drawn up by the European Commission.

‘Hobbit’ Discoverer Dies

Archaeologist **Michael Morwood**, co-discoverer of *Homo floresiensis*, the “hobbit” of Indonesia, died on 23 July after battling cancer. He was 62. In 2003, Morwood, then of the University of New England (UNE) in Armidale, Australia, and colleagues announced finding the skeleton of a woman barely a meter tall and with a tiny brain, who



Morwood

THEY SAID IT

“Changing the language we use to diagnose various lesions is essential to give patients confidence that they don’t have to aggressively treat every finding in a scan.”

—Oncology surgeon Laura J. Esserman to *The New York Times*, on a report she co-authored in *The Journal of the American Medical Association* that recommends restricting “cancer” to lesions likely to be lethal if left untreated.

lived 18,000 years ago on the Indonesian island of Flores. The find was controversial: Some scientists suggested that the skeleton was a diseased *H. sapiens*, not a new species (*Science*, 10 August 2007, p. 740). Most claims for specific diseases that might have afflicted the hobbit have since been discredited, although researchers have yet to uncover *H. floresiensis* bones anywhere other than Liang Bua Cave on Flores.

>>

Random Sample

NASA Investigates *Space Vikings*

For Ved Chirayath, an aeronautics and astronautics graduate student at Stanford University in California, an online photo project that involved NASA researchers dressed as Vikings was just a creative way to promote space science. Chirayath, an amateur photographer who also works at NASA's Ames Research Center (ARC) on compact research satellites known as "CubeSats," never suspected that his fanciful image would put him in the crosshairs of a government waste investigation.

CubeSats, he says, reminded him of Viking explorers who "travelled farther and saw more in much smaller ships than had been used before their time." That connection inspired his *Space Vikings* photos, snapped with the participation of a few costumed NASA researchers as well as a living history group called the Vikings of Bjornstad.

But on 10 July, Senator Charles Grassley (R-IA) wrote to NASA chief Charles Bolden, asking him to investigate whether Chirayath's photos involved the possible misuse of ARC funds and staff time. Grassley asked Bolden to help him "better understand the participation of NASA



employees and resources in this for-profit photography exhibit."

Chirayath says that his effort was strictly not-for-profit and didn't involve ARC funds—and NASA News Chief Allard Beutel says the agency concluded that no taxpayer funds were used for *Space Vikings*. "The employees were on their time, not on work time."

The flap left Chirayath perplexed. "NASA can't afford to promote their missions in this way and this is partly why I started this project," he says. And that's ironic, he adds, because "more was probably spent in taxpayer employee man-hours investigating me, my exhibition, and those involved than it might have cost" to produce the photos professionally.

>> NEWSMAKERS

Morwood got his start studying Australian rock art. He returned to such analyses in recent years—while continuing to hunt for hobbit bones—at the University of Wollongong in Australia.

Morwood "embodied what archaeology in a university department should be about—both creating and passing on knowledge about the past," says archaeologist and colleague Iain Davidson of UNE. "His ability to work with [indigenous peoples] and with Indonesian scholars is testament to his humanity."

FINDINGS

A New Twist on the Oldest Radiation

Astrophysicists have spotted swirls in the afterglow of the big bang—the cosmic microwave background (CMB). The result suggests that scientists are closing in on a major discovery: gravity waves that rippled through the infant universe during a faster-than-light growth spurt called inflation.

The CMB's microwaves are polarized, and grav-

ity waves should produce swirls, or B-modes, in the polarization pattern in the sky. But B-modes can also arise when gravity from matter in the foreground distorts the CMB. Duncan Hanson, an astrophysicist at McGill University in Montreal, Canada, and colleagues working with the South Pole Telescope teased out those other B-modes starting with an estimate of the mass distribution in the universe from other data.

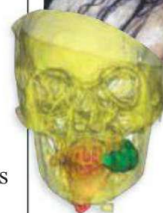
"I take it as a hopeful sign that we can get to the gravitational-wave signal," says Charles Bennett, a cosmologist at Johns Hopkins University in Baltimore, Maryland. Several teams are racing for that prize, including one with European Space Agency's Planck spacecraft. Planck researchers "are pushing very hard on this," says David Spergel, a cosmologist at Princeton University. "They hear footsteps."

'Llullaillaco Maiden' Was Drugged Before Sacrifice

The 500-year-old, naturally mummified bodies of three children, probable victims of an Incan sacrificial ritual known as *capacocha*, were found in 1999 atop Argentina's Llullaillaco volcano. It now appears that regular use of coca and alcohol might have played a more-than-ceremonial role in their deaths, according to a new study in the *Proceedings of the National Academy of Sciences*.



Sacrificed. Radiological scans (left) show the maiden was chewing coca leaves when she died.



Most of what scientists know about the lives of the children—a boy and girl about 4 to 5 years old and a 13-year-old female called the Llullaillaco Maiden—comes from their hair. Key metabolite levels measured in the Maiden's hair suggest that her coca use peaked 6 months before she died, while her alcohol consumption skyrocketed in her final weeks. The boy and the girl also ingested the two drugs but in much smaller amounts.

While other high-altitude Incan mummies show signs of head trauma, the Llullaillaco mummies appear to have died peacefully. The alcohol may have sedated the Maiden in the weeks before her death, and also impaired the shivering reflex, hastening her death of exposure, notes Andrew Wilson, an archaeologist at the University of Bradford in the United Kingdom.

<http://scim.ag/cocamummy>

STRUCTURAL BIOLOGY

Is High-Tech View of HIV Too Good to Be True?

Structural biologists over the past 15 years have used increasingly sophisticated tools to offer fantastically detailed views of HIV's surface proteins. On 11 June online, the *Proceedings of the National Academy of Sciences (PNAS)* published the most exquisite yet, showing the molecular architecture of the proteins in the finest resolution ever. It comes from a prominent research group and promises to guide HIV vaccine design for years. If it is right.

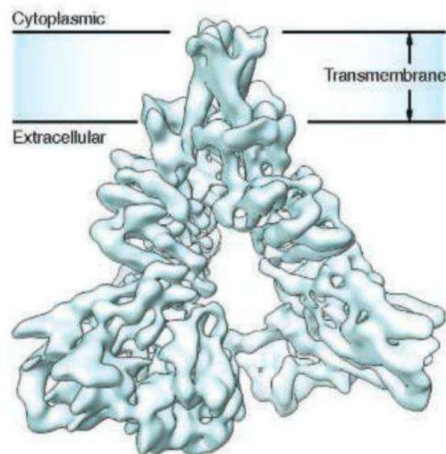
Several respected HIV/AIDS researchers are wowed by the work. But others—structural biologists in particular—assert that the paper is too good to be true and is more likely fantasy than fantastic. “That paper is complete rubbish,” charges Richard Henderson, an electron microscopy pioneer at the MRC Laboratory of Molecular Biology in Cambridge, U.K. “It has no redeeming features whatsoever.”

That scorching assessment is especially startling given that the work comes from the lab of Joseph Sodroski, a virologist at the Dana-Farber Cancer Institute in Boston. He has published nearly 400 papers, including more than three dozen in *Science*, *Nature*, and *Cell*. Sodroski is low-key and anything but a lightning rod for criticism. Yet during the past year, *Science* has learned, these three top-tier journals and at least one other rejected various iterations of his lab's findings using a technique called cryo-electron microscopy (cryo-EM), which visualizes individual proteins after rapid freezing at liquid nitrogen temperatures. Henderson, who says that he wrote critical reviews of the work for two publications, is leading a pack of structural biologists who are in an uproar that *PNAS* published it.

Sodroski and first author Youdong Mao, a postdoc in Sodroski's lab, are not yielding any ground. “We stand behind our published work,” they write in an e-mail to *Science*. “If anyone has specific scientific issues with our methods, results or interpretations, they should voice their concerns in the proper scientific forums. We will respond to such concerns in the peer-reviewed scientific literature.”

The *PNAS* paper shows a 6-ångström

view of HIV's surface proteins gp120 and gp41, which are attached to each other and appear in clusters of three called trimers. In contrast to old-fashioned electron microscopic methods, cryo-EM allows visualization of the trimers without addition of stains that can distort shapes. If the results hold, Sodroski, Mao, and co-workers have unveiled the sharpest view of the trimers ever captured in the precise configuration that the immune system sees them. This could lead to



Unresolved debate. Is this 6-Å resolution structure of HIV's surface proteins jutting through a cell membrane real?

new insights about how antibodies capable of stopping HIV bind to the virus.

Most of the structural biologists and HIV/AIDS researchers *Science* spoke with, including several reviewers, did not want to speak on the record because of their close relations with Sodroski or fear that they'd be seen as competitors griping—and some indeed are competitors. Two main criticisms emerged. Structural biologists are convinced that Sodroski's group, for technical reasons, could not have obtained a 6-Å resolution structure with the type of microscope they used. The second concern is even more disturbing: They solved the structure of a phantom molecule, not the trimer. “Preparation of cryo-EM specimens that are capable of producing high-

resolution structures of membrane proteins in solution is challenging,” says structural biologist Sriram Subramaniam, a cryo-EM specialist at the U.S. National Cancer Institute in Bethesda, Maryland, who has published 9-Å views of HIV trimers. Like Henderson and other structural biologists interviewed, Subramaniam doubts that Sodroski's group had any HIV particles in their samples.

The essential problem, they contend, is that Sodroski and Mao “aligned” their trimers to lower-resolution images published before, aiming to refine what was known. This is a popular cryo-EM technique but requires convincing evidence that the particles are there in the first place and rigorous tests to ensure that any improvements are real and not the result of simply finding a spurious agreement with random noise. “They should have done lots of controls that they didn't do,” Subramaniam asserts. In an oft-cited experiment that aligns 1000 computer-generated images of white noise to a picture of Albert Einstein sticking out his tongue, the resulting image still clearly shows the famous physicist. “You get a beautiful picture of Albert Einstein out of nothing,” Henderson says. “That's exactly what Sodroski and Mao have done. They've taken a previously published structure and put atoms in and gone down into a hole.” Sodroski and Mao declined to address specific criticisms about their studies.

Beatrice Hahn, a virologist at the University of Pennsylvania and a member of the National Academy of Sciences, served as editor of the *PNAS* paper, meaning that she selected the referees and oversaw the review process. “This is about the hottest-button issue I've encountered in my 30-year career,” says Hahn, who is well-known for her studies of HIV's origins. “For the past almost 2 years now, I've watched some of the criticisms and the responses and both sides are certainly reasonable.” Hahn decided that it was time to intervene. “When there's a potential leap



Imaginary image. Improperly used technique from cryo-EM can recover Einstein from white noise (right).

that's either totally wrong or right, that information has to be made public [in a journal]. All sorts of people can look at it and make up their minds."

Hahn acknowledged that she is not versed in the complicated cryo-EM technique enough "to really judge one way or the other" whether the criticisms are legitimate. She says Sodroski told her that Mao previously used cryo-EM to study graphite and routinely pieced together suboptimal images, which may help explain how he achieved a higher resolution than specialists who study only biological samples. She insists that the paper received a bona fide peer review at *PNAS* and urges people to remember that Sodroski has a record of producing credible work. "My gut tells me that Joe is right."

Bette Korber, an immunologist at the Los Alamos National Laboratory in New Mexico who uses models of HIV trimers to help design vaccines, admires how Sodroski is handling the criticism. "He is systematically addressing the points raised in reviews or in conversation with other scientists in the field," Korber says. "I've known Joe for most of my professional career, and find him to be a person of great integrity, and I am optimistic he will continue to be able address the concerns of others as research advances in this area."

Sodroski impressed many HIV/AIDS researchers in March 2012, when he first presented the work at the Conference on Retroviruses and Opportunistic Infections, a meeting Hahn helps organize. By then, the work had already sparked skepticism in cryo-EM circles. When he first shopped a paper claiming a 2.6-Å structure—near atomic level—Henderson, for one, was incredulous. "If true, several synchrotrons in the world and cryo-EM specialists would all be out of business," he says, noting that he refereed conflicting reviews of this version of the paper for *Nature*.

Last August, Sodroski's group published an 11-Å version of the trimer online in *Nature Structural & Molecular Biology*, a resolution more plausible to Henderson and others. But when Henderson was later asked by the same journal to referee a paper describing the 6-Å trimer, he wrote an extensive critique. The *PNAS* paper, he says, is revised only slightly from the version he read. "I was shocked," Henderson says. "They completely ignored my five pages of critiques."

Hahn says she has yet to see an experiment that proves Sodroski and Mao made a mistake. "Ultimately," she says, "the biology has to decide who's right and who's wrong."

—JON COHEN



SOCIAL SCIENCE

Study Links Climate Change And Violence, Battle Ensues

In many large cities, police know that a hot summer will be a busy time, as murder and mayhem spike with the temperature. Some social scientists say that the same pattern could hold globally. Along with wilted harvests and a loss of biodiversity, they say, climate change could lead to escalating violence. Now, a study published online this week in *Science* tries to quantify the increase (<http://scim.ag/SHsiang>). Based on dozens of published studies correlating extreme weather and human conflict, it concludes that warmer temperatures and more extreme rainfall patterns could boost interpersonal violence by 16% and group conflicts in some regions by 50% by 2050.

"We were conservative and the result is still clear," says econometrician Solomon Hsiang, the paper's lead author, who will soon join the University of California, Berkeley. Thomas Homer-Dixon, a political scientist at the Balsillie School of International Affairs in Waterloo, Canada, who has studied climate change and conflict since the 1990s, agrees. "This study, precisely because it's an extremely carefully done meta-analysis, will push the debate a big step forward."

The study's critics, however, aren't impressed—and the paper is adding fuel to a long-standing dispute over the possible links between climate and conflict. One problem, they argue, is that the study conflates weather with climate. Another is that the researchers may have based their conclusions on a biased subset of studies. "They are more optimistic and confident in their

results than I would be," says Andrew Solow, a statistician at the Woods Hole Oceanographic Institution in Massachusetts.

Standing in the crossfire is Hsiang, who says that when he first got interested in the issue 2 years ago while finishing his Ph.D. dissertation at Columbia University, "I had no idea that I was stepping into the middle of a debate that has been raging for 20 years."

The climate-violence connection has long intrigued scientists. Psychological studies have shown that people become more aggressive when the temperature becomes uncomfortably hot, and higher temperatures are known to correlate with higher urban homicide rates. Researchers have also fingered extreme precipitation—floods and droughts—for promoting violence.

In his dissertation, Hsiang explored a possible association between conflict and shifting weather patterns due to El Niño events in the Pacific Ocean. The work led to a 2011 *Nature* paper that demonstrated that conflicts spiked in areas affected by El Niño. A year later, he began collaborating on a broader study with Marshall Burke and Edward Miguel, both economists at Berkeley.

They started with "about 1000" papers that touched on the topic, which they whittled down to several hundred that had sufficient data for analysis. Many of these were cross-sectional, comparing rates of violence between places that have different climates. "That is a very tempting approach," Hsiang says. "The problem is that when we compare populations in very different locations, they

CREDIT: AGENCY VU/GETTY IMAGES

Heated conflict. Researchers debate whether climate change spurs violence, such as the conflict in Sudan's Darfur.

are different in ways that we can't capture in a model." Differing histories and cultures, for example, may better explain patterns of violence than differences in climate.

To avoid that problem, Hsiang and his co-authors restricted themselves to 61 longitudinal studies that collected conflict and weather data from the same places over time. The "mountain of data" covered a range of scales, from studies of interpersonal violence such as assault, murder, and rape, to group- or state-level conflicts such as riots and wars. The studies spanned all regions of the world and stretched back 10,000 years. A majority had been published since 2009.

A clear pattern emerged. When the average precipitation or temperature in a place strayed from its average seasonal value, violence tended to increase. Interpersonal violence increased by 4% for an increase of 1 standard deviation away from the average (for example, a New York City summer about 3°C above average). And the effect was stronger for intergroup violence, with a 1 standard deviation difference translating into a 14% increase in the frequency of such conflicts. In some regions, those numbers could mean an increase of up to 50% in the frequency of violence by 2050, Hsiang and his colleagues note, if global temperatures increase by the 2 to 4 standard deviations projected by mainstream climate scenarios.

That forecast is unfounded, argues Halvard Buhaug, an economist at the Peace Research Institute Oslo, because the study suffers from "selection bias." The authors ignored some data in the selected papers, he says, and "more worrisome," appear to have used data "that return the strongest effects." Hsiang says they dealt with the potential for bias "head on" with statistical analysis, but found little.

The selected papers may have also confused "single sharp" weather events such as heat waves with longer term climate shifts, Solow says. He points out that in sub-Saharan Africa, there is no doubt that climate change has been unfolding over the past several decades. But over the same period, "the overall rate of civil conflict has declined."

Hsiang's study "is an amazing compilation and analysis of data" that "makes you think," says archeologist Richard Potts of the Smithsonian Institution in Washington, D.C. But he's not convinced, he adds, and "it does little to end the impasse" among those who foresee more violence in a warmer world, and those who are cool to the idea. —JOHN BOHANNON

DRUG DEVELOPMENT

Corruption and Research Fraud Send Big Chill Through Big Pharma in China

SHANGHAI, CHINA—The widening bribery investigation engulfing the Chinese branch of drug giant GlaxoSmithKline (GSK) focuses on the tactics of its sales people. But it also adds to a perfect storm of scandals involving multiple companies that could jeopardize China's rise as a center for global pharmaceutical R&D.

On 11 July, the Chinese government accused executives at GSK China of bribing officials, hospital employees, and doctors to promote or sell GSK drugs. Four Chinese GSK executives, along with at least 18 other employees and medical personnel, have been taken into custody, according to state press reports. Last week, GSK replaced its general manager for China and acknowledged in a statement that "Certain senior executives ... appear to have acted outside of our processes and controls." The company also pledged to lower its prices in China.

The accusations come as China—particularly Shanghai—is emerging as a research hotspot for big pharma. Helping propel China's rise as a global research hub, officials encourage R&D investment in return for market access. With the corruption probe at GSK coming on the heels of other scandals directly connected to research, observers say that the industry should brace for harsh consequences. "Whatever happens to GSK will

have a huge impact on pharmaceutical industry investment in China, on Chinese scientists' reputation, and on the future development of medicine," says a scientist familiar with R&D at GSK who did not wish to be named. Companies may consider stricter controls on big pharma's R&D operations in China. But no one expects companies to turn tail. "Exiting China is not in the cards," says Benjamin Shobert, director of the Rubicon Strategy Group in Seattle, Washington. "And China knows this."

The nature of the allegations against GSK, which involve funneling bribes through a Shanghai travel agency, surprised few in

the industry. GSK is "certainly not an isolated case," says Yanzhong Huang, a global health fellow at the Council on Foreign Relations in New York City. Last December, the U.S. Securities and Exchange Commission charged Eli Lilly with violating the Foreign Corrupt Practices Act by bribing doctors on the Chinese state payroll with spa treatments, jewelry, and money. Industry insiders say that multinationals simply take cues from domestic counterparts, and lavish drug-sales inducements are a familiar practice in Western countries, such as the United States.

But other scandals have directly hit the growing drug R&D business in China. As big pharma closes or scales back research facilities in the United States and Europe, many companies are opening up shop in Shanghai or Beijing, attracted by an ample scientific labor force and a large patient pool for clinical trials (*Science*, 27 July 2007, p. 436). GSK is a giant on the Chinese scene, having spent more than \$163 million on R&D

Footholds in the Far East

	R&D center established	Investment	Staff
GSK	2007, Shanghai	\$163 million over 20 years	400
Pfizer	2005, Shanghai	\$150 million	700
Novo Nordisk	2002, Beijing	\$40 million in 2010	100
AstraZeneca	2007, Shanghai	\$35 million since 1996	
Merck	2011, Beijing	\$1.5 billion pledged for 2011–2016	N/A
Roche	2004, Shanghai	N/A	110

Pharma mecca? Despite trimming Western operations, multinationals have lavished funding on R&D centers in China.

in China in the past 2 decades. Its Shanghai center, launched in 2007, focuses on neurodegeneration. Other big players include Pfizer, which has invested more than \$150 million in its Shanghai center, and Merck, which in 2011 pledged to spend \$1.5 billion on R&D in China over 5 years (see table, above).

In both Chinese and multinational outfits, an emphasis on quick results may tempt researchers to cut corners. "We spend a lot of time teaching people how to follow the guidelines," says Zhai Yifan, CEO of HealthQuest Pharma, a biotech startup in Guangzhou. The potential for negligence was thrust into the spotlight in June, when GSK

fired its R&D head here for misrepresenting data in a preclinical study on an experimental drug for multiple sclerosis. The company halted phase I clinical trials of the drug and has asked *Nature Medicine* to retract a 2010 study. An internal audit leaked to *The New York Times* last week suggests that problems with research conducted by GSK's Shanghai center may have extended to other drugs. Then last month, media reports based on U.S. Food and Drug Administration (FDA) documents alleged that clinical trial data from China for the drug Eliquis, a blood thinner developed by Bristol-Myers Squibb and Pfizer, were marred by errors and fraud. The errors resulted in a 9-month delay for FDA approval, which was granted last December.

Observers say that the incidents could give ammunition to skeptics of China's R&D potential. "There are people in the U.K. and the U.S. who will say 'See? I told you,'" says the unnamed scientist. But China-based R&D is critical to big pharma's future business. Chronic diseases such as diabetes and hypertension are on the rise in China's large, rapidly aging, and increasingly affluent population. Drug companies emphasize diseases common in Asia in their China research operations: Novartis's Shanghai center focuses on hepatitis B and C, while Astra Zeneca's Shanghai shop studies liver and gastric cancers. An army of efficient contract research organizations in China eases the path for drug development. Moreover, Chinese law demands

a presence: To register new drugs in China, companies must first conduct trials with local patients. The R&D operations are propelled by burgeoning revenue in China, which grew from \$4 billion in 2006 to \$10 billion in 2011 for the top 10 pharmaceutical multinationals, according to the consulting firm McKinsey.

That growth may be about to flatten. As part of its healthcare reforms, the central government has mandated price cuts on hundreds of drugs, many produced by big pharma. And revised regulations now allow for compulsory licensing, which would allow domestic manufacturers to make generic versions of patented drugs on a case-by-case basis—a tactic the government has not yet invoked.

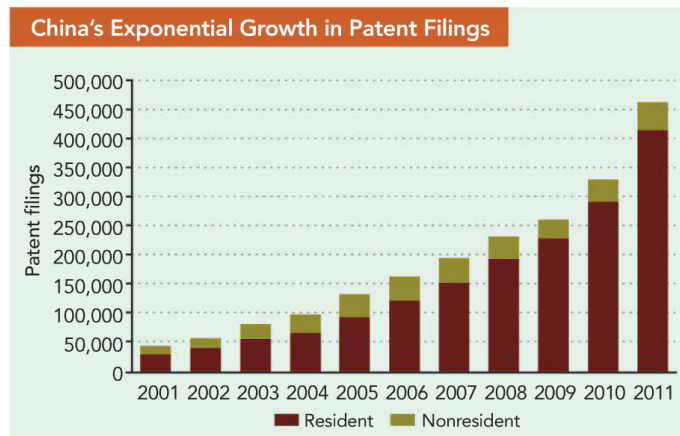
At the same time, the government has been aggressively promoting domestic drug R&D (*Science*, 3 July 2009, p. 21). The Major New Drug Innovation Program, launched in 2009 to boost drug development by establish-

ing innovation bases around China, enjoys more than \$1.5 billion in central government funding, along with \$3 billion from local governments and industry, according to Lux Research. And the government subsidizes patent fees for domestic companies, fueling a rapid increase in filings (see graph, below). Multinationals "have to prepare for the prospect that one day they will compete with their Chinese counterparts as equals," Huang says.

Some of China's hopes for its domestic drug companies may be unrealistic. The central government has set a goal of developing 30 innovative drugs by 2015, and another 70 by 2020. "It's unlikely that we will see a great drug" by 2015, says Hu Zhuohan, president of the Research Institute for Liver Diseases here who has served on China's State Food and Drug Administration committees considering new drug applications. While the injection of cash should eventually yield

results, Hu says, "what drugs come out is not directly correlated with how much money is put in."

Meanwhile big pharma, chastened by the corruption probes, is cleaning up its operations. Most clinical centers now scrupulously follow FDA Good Clinical Practices regulations, says Zhai, the Healthquest CEO: "The early days of making up data" are over, she insists. Zhai believes that the scandals have delivered a "wake-up call" to improve oversight and management in China. "In the long run," she says, "it's a good thing." —MARA HVISTENDAHL



Patent boom. Favorable policies have fueled a surge in resident patent filings, many by pharmaceutical or biotechnology firms.

SCIENTIFIC EXCHANGE

Marine Studies Show Potential for U.S.-Cuban Collaboration

CAYO COCO, CUBA—Dense mangroves eclipse the midday sun in this inlet off northern Cuba's La Redonda Lake, as a team of six ecologists steps off an idling speedboat. They are here to gather baseline data on the mangrove ecosystem and focus on a local mystery—the disappearance of the large-mouth black bass (*Micropterus salmoides*). Once prolific, it has been vanishing from this mangrove lake over the past few years. Elevated water temperature or other climatic factors are possible causes, but the team says nothing has been ruled out.

Adán Zúñiga Ríos, leader of the project, wonders whether something similar has happened in South Florida, more than

150 kilometers away, home to almost identical mangrove thickets. "Ecosystems know no country borders," says Ríos, director of the Center for Coastal Ecosystems Research (CIEC), based in this coastal town. Yet Zúñiga has been unable to work with U.S. scientists to gather parallel data in Florida.

"If we were dealing with any other two countries," collaboration wouldn't be so difficult, he says. But this is Cuba, and for 51 years the U.S. economic embargo has made interactions cumbersome, if not impossible. But change may be coming. Early this year, Cuba eliminated the "white card," a type of exit visa required of anyone who wants to travel abroad. Other barriers

that keep professionals in both countries from visiting one another—especially doctors and scientists—might be relaxed soon, says Daniel Whittle, a conservationist and Cuba program director for the Environmental Defense Fund (EDF). Whittle says that he was encouraged by what he heard from U.S. and Cuban officials while hosting a workshop on fisheries in Cuba last month. Whittle says that no formal proposals are on the table, but both governments seem to be opening up to more "people-to-people" exchanges—not just ceremonial events in Havana.

The mangroves are just one area of potential cooperation; there's a huge environmental



Deep fieldwork.
A research team gathers data on wildlife in a mangrove ecosystem near Cayo Coco on Cuba's north coast.

overlap between the two neighbors. "There is a lot we can learn from each other," says Peter Agre, 2003 Nobel laureate in chemistry and former president of AAAS (*Science's* publisher), who has traveled to Cuba three times in recent years to promote exchange.

There's a lot the two countries could learn to avoid, too. "A shared environment means shared risks," says Whittle, whose organization has worked with groups like CIEC since 2001 on marine conservation. Offshore oil exploration and commercial fishing are two current areas of risk. The Obama administration has taken small steps toward easing scientific exchanges in some areas, like oil. "It's in our national interest to get this right," Whittle says, although "we're still a long way off."

Ties between U.S. and Cuban scientists date back to the 19th century, but most exchanges ended in 1961, when the John F. Kennedy administration cut ties with Cuba and subsequently imposed an economic embargo after socialist leader Fidel Castro rose to power. Scientific exchange improved a bit in the 1970s when the Smithsonian Institution in Washington, D.C., signed an agreement to reestablish limited ties with the Cuban science academy. During the Clinton administration in the mid-1990s, scientists launched other collaborations, including an ongoing project led by the New York Botanical Garden to help Cuba identify vulnerable plant species.

But the barriers are still daunting. "The basic stuff is hard to get done," says James A. Powell, founding director of the Sarasota, Florida-based Sea to Shore Alliance. His group was invited by the University of Havana's Center for Marine Investigations (CIM) to participate in a study of manatees. Since 2003, Powell says, they have found movement between manatee populations in Cuba and Florida that was previously

unknown. Researchers rely on a massive photo database to track individual animals, identified by unique scars caused by being struck by boats. Powell recalls one manatee that he photographed himself in Florida in 1978; it was later sighted on a beach in Cuba, in 2007.

Powell says that researchers in Cuba have gathered DNA from carcasses and live animals suggesting that the predominant manatee in Florida may be "more closely related to the Antillean manatee found in Cuba than we thought before." A key achievement, he says, has been establishing causes of death, including CIM work showing that many of Cuba's manatees were drowning in fishing trawls. The government has taken steps to limit fishing in manatee areas, and the population may begin to recover. But without the embargo, Powell says, "we would be able to build on this strong foundation ... and develop a more comprehensive conservation program."

The embargo guidelines set by the U.S. Treasury Department's Office of Foreign Assets Control allow U.S. scientists to fund their own work in Cuba, but not to support work by Cubans. U.S. researchers may not donate equipment or purchase anything permanent in Cuba—be it a boat or office space. Powell says, "All my equipment—including binoculars—has to be registered and approved" by the U.S. government before being taken to Cuba.

The rules constrain activities, too, says Fernando Bretos, a Miami, Florida-based biologist with the Ocean Foundation who has studied sea turtles in Cuba since 1999. When Bretos wanted to track migration patterns, he waited months for authorization to take small satellite tags into Cuba. Bretos then learned that he couldn't teach Cuban scientists how to affix the tags to animals without another license for "sharing of

information." Dreading the paperwork and the likely 1-year wait, Bretos decided to put the tags on himself.

More than science could be at risk. Since 2011, Cuba has been opening up its economy, and experts worry that burgeoning commercial fishing, for example, could affect species that migrate to U.S. waters, such as grouper, snappers, and tarpon. Pollution and coastal development could also affect endangered animals shared by the Florida Keys—manatees, sharks, and sea turtles.

Cuba's plans for its undersea oil reserves could pose a bigger threat. Little has been extracted so far—Cuba has just one working rig, off its central northern coast. But some think that the oil reserves could be vast, and just one blowout could be catastrophic. "*Deepwater Horizon* was a game changer," says EDF's Whittle, referring to the 2010 oil spill in the Gulf of Mexico. It forced everyone to recognize that the ocean is a shared ecosystem, and in 2012 the U.S. State Department and the Cuban Ministry of Foreign Affairs started discussions that could soon lead to an agreement outlining procedures and protocols in the event of a spill, Whittle says.

Scientists hope that the détente can be extended. In February, a group of U.S. scientists wrote to President Barack Obama noting that "the process for carrying out environmental projects with Cuba remains daunting." They asked that he continue streamlining processes for securing equipment and travel licenses for work in Cuba, as well as ease regulations that limit the use of U.S. funding in Cuba. While there was no official response, several of the letter's signatories met with White House and State Department staff members to discuss their recommendations, and they say there's hope that President Obama's second term will bring about further openings.

Cuba has its own constraints, scientists say: too much red tape and too little money. All projects must be vetted through government agencies, often waiting years to clear the bureaucratic labyrinth. Once a project is under way, there's no guarantee of continuity.

Yet Cuban scientists can contribute knowledge and enthusiasm. On his visits to Cuba, Agre says that he was impressed by "so many bright-eyed young people passionate about wanting to do science." He adds, "Politics is never an issue. ... We just start talking science and that's it, connections are made." He and others hope that can become the norm.

—JEAN FRIEDMAN-RUDOVSKY

Jean Friedman-Rudovsky is a freelance writer.

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BIOINFORMATICS

Top Contenders Blast Pentagon's New Bioterror Detection Prize

A \$1 million prize in DNA analysis from the Department of Defense has yet to name a winner, but some top contenders are already saying that the contest is unfair. The Defense Threat Reduction Agency's (DTRA's) Algorithm Challenge tasked competitors with finding a radically faster and more accurate way to identify the species and genes in raw DNA to spot potential bioterror threats. Envisioned as a convenient, crowdsourced solution to a key bioinformatics problem, the contest proved so difficult that only three of 103 competitors made the final cut. As participants await the announcement of a winner in September, many claim that unclear ground rules and an unfair scoring system make it unlikely that the best quality work will prevail.

"The way they organized the competition and the way they scored it was just horrible," says David Ainsworth, a bioinformatics Ph.D. student at Imperial College London, whose team is still in the running. "I think it's unlikely that the best algorithm will win," says bioinformaticist Steven Salzberg of Johns Hopkins University in Baltimore, Maryland. Salzberg did not participate, but a Ph.D. student and a bioinformatics engineer in his lab narrowly missed the cut.

Organizers admit that the contest was tough and say that figuring out where to set the bar was tricky. "You don't want to make it so high that nobody can win, but you don't want to set the bar so low that you have 200 people tied for first place," says Christian Whitchurch, the project manager. But he is confident that the contest will yield a valuable program. Independent review panels helped develop the challenge and will approve the final decision, Whitchurch says.

In January, DTRA, a Pentagon agency charged with a wide range of security tasks, laid out the challenge: Develop a program to detect potentially dangerous organisms and their individual genes in DNA samples in less than an hour—a vast improvement over today's capabilities.

To manage the contest, DTRA chose InnoCentive, a company that hosts online

challenges. This is the largest prize that DTRA has offered, and it represents a departure from the agency's normal way of doing business. By accepting entries from anyone who registered online, DTRA could tap the knowledge of a diverse group of scientists without the red tape of the bidding and contracting process, Whitchurch says.

For participants, competing required a high-risk investment of time up front, but it also eliminated the traditional barriers of the grant process. "Having the skill to actually solve something and having the skill to write a proposal to get the money—they're two different skill sets," says Daniel Huson, a bioinformaticist at the University of Tübingen

working, participants could submit their results for scoring along the way, but some say that they were mystified by the feedback. Derrick Wood, a computer science Ph.D. student at the University of Maryland, College Park, who did not qualify for evaluation, complains that he lost points when he did not name the specific strain of an organism he had identified, but received no extra points when he did add strain information in other cases. Ainsworth, too, was frustrated. "We spent the last month trying to get the [scoring] algorithm to tell us that we've done well, instead of actually doing the proper science to produce a good result."

When no one qualified during the first 4 months of the challenge, DTRA pushed back the 31 May deadline and relaxed some of the rules. As contestants continued to struggle, the agency bumped its deadline to 14 July. "I admit that I was nervous when we only had a month to go and no one had met the threshold," Whitchurch says.

Less than a week before the cutoff, participants were encouraged to team up to improve their scores. There was "a flurry" of desperate e-mails among teams in the last 24 hours, says Ainsworth, who partnered with another solo competitor in the nick of time. A newly formed team could use outputs from different algorithms on different data sets, swapping results to piece together a passing score. "It's just a completely artificial way to game the system," says contestant Robert Edgar, a self-funded computational biologist who did not make the cut.

The three qualifiers have now uploaded all of their code for final evaluation. The winning program must meet both speed and accuracy requirements on the original data sets and a brand-new DNA sample. If no team meets these requirements, some of those who had been disqualified may be considered, Whitchurch says. The organizers also plan to release a detailed explanation of the selection process after a winner is announced.

Ainsworth and his teammate tweaked one of their algorithms before submitting, but have no idea if it can handle all nine original data sets. They'll find out in September, when the results of the evaluation phase are announced. "I'm just treating it as a lottery now," he says.

—KELLY SERVICK



Contested. Some scientists competing for a \$1 million prize to detect bioterror threats, such as the 2001 anthrax attacks, say the rules are unclear and the scoring unfair.

gen in Germany, whose team is among the qualifiers. Enticed by the million-dollar prize, roughly 2700 people signed up for the challenge, and 103 competitors submitted work, including both individuals and teams. Huson and his teammates dropped everything to devote 17-hour days to the project.

The entrants received nine data sets, each containing a mix of genetic code from unknown sources. They submitted results to an automated scoring system, which sent back an accuracy reading. To qualify for the evaluation round, where programs will be scored and the \$1 million algorithm selected, they needed to reach a certain accuracy level on all nine data sets.

To check how well their algorithm was



Venturing Back Into Colombia

In rural areas where guerrillas and drug traffickers have long been in control, researchers are now on the scene—but so are cattle ranchers, miners, and palm oil planters

BOGOTÁ—Douglas Daly, a U.S. expert on Amazonian plants, vividly recalls the collecting trip that he took to Colombia in 1987. Jostling along a rutted track in their Jeep, he and three colleagues ran into a group of nervous men with AK-47s. The guerrillas demanded to know who the intruders were and why they had so much gear. Daly sweated through the grilling, pretending to be Brazilian. He got by but says, “It was a wake-up call. We got out of there and didn’t go back.” Not for 20 years.

Now Daly, the curator of Amazonian botany at the New York Botanical Garden in the Bronx, is going back—again and again. In April, he made his third scientific expedition to Colombia since 2010, collecting 500 plants in 2 weeks, “four or five of which are new to science,” Daly says. “That is probably the best haul I’ve had in that short a time.”

Colombia is a country of huge biodiversity. It ranks first in the world in number of flowering plants, second in birds,

and sixth in mammals, according to the Food and Agriculture Organization of the United Nations. The twin Andes mountain ranges running north to south carve spectacular transitions between the jungle and the two ocean coasts where species flourish and evolve. Yet the fauna and flora are not as well described as those in neighboring countries. A decades-long civil conflict, pitting guerrillas in remote bases against the government, turned much of the countryside into a no-go zone for science.

But over the last decade Colombia’s government has pursued a successful security campaign to take back roads and territory; it is now engaged in peace negotiations with the guerrillas. Conflict has been winding down, drawing biologists back to the field. They’ve made a surge of discoveries, describing new species of frogs, birds, even monkeys. “It’s true,” says Brigitte Baptiste Ballera, director of the Alexander von Humboldt Biological Resources Research Institute, in Bogotá.

“The map for science has changed.”

But scientists aren’t the only ones racing back. Gold miners, oil companies, and cattle ranchers—also excluded by the conflict—are also taking advantage of the spread of peace. Along with opportunities for study, researchers see new and unprecedented risks to biodiversity.

Daly says that he experienced a swirl of contradictory emotions in April during a visit to the department of Guaviare, where he collected leaves, branches, and green fruit from an as-yet unnamed tree. “To go to a habitat and find [that] the dominant tree has no name—well, that is pretty intense,” Daly says. Yet on a flight over the country, he says, mostly what he saw from the window was the spread of cattle ranches and African oil palm plantations.

It comes with the job

In the late 1980s, as rebels gained ground, foreign researchers began to decamp from Colombia. But many local scientists found ways to continue their fieldwork. Marxist rebels in many cases regarded professors as sympathetic. With the right contacts, some scientists spent months in the jungle, although the work had its hazards.

CREDIT: COURTESY OF PAOLA PEDRAZA/NEW YORK BOTANICAL GARDEN

Botanical hunt. Colombian and U.S. field researchers trek into the Andean forests of Las Orquídeas National Park.

"I knew [the guerrillas] pretty well," says Andres Etter, a forest expert at the Javeriana University in Bogotá. "But starting around 2001 we became targets for kidnapping, too."

John Lynch, an American frog specialist at the National University of Colombia in Bogotá, was kidnapped twice, each time released after a day or two. Once he was held in a wooden cage. "It was the cost of doing fieldwork," he says. By the late 1990s, right-wing paramilitary groups emerged as a new threat, along with well-organized cocaine producers. Tens of thousands of people were killed, including 10 employees of Colombia's national park service while on duty. The respect for professors ended, says Baptiste, who was threatened by a paramilitary group. "So the universities stopped going in and stopped doing fieldwork."

Field stations shut down, and many top students left for Mexico or Venezuela. "Science kept going, but at a very slow, painful pace," says Paola Pedraza, assistant curator at the New York Botanical Garden, and a Colombian. Without adequate funding or expertise, Colombia lagged, she says, especially in DNA-based phylogenetics and bioprospecting. Colombia became a blank spot on modern maps of species. At the Missouri Botanical Garden, a large U.S. herbarium in St. Louis, the number of new plants sent in from Colombia between 2000 and 2009 was 85% lower than in the 1980s, according to Pedraza, who analyzed submissions.

The information gaps made it more difficult to evaluate risks from climate change and identify biodiversity hot spots that need management. This work is critical for Colombia because its high-altitude, cool-weather species will have

Discovery of a New Titi Monkey

FLORENCIA, COLOMBIA—Javier García was an undergraduate when he made the find of a lifetime in 2008—a new primate now listed as *Callicebus caquetensis*, the red-bearded titi monkey. Biologists had heard that such a creature might live in this remote hot spot, García's home province of Caquetá. But they were reluctant to venture into a place where rebels and paramilitaries battled over territory and U.S.-financed aircraft sprayed herbicide on coca plants (see main story, p. 450).

Discovering a new species at age 22 launched García as a conservationist. He is working on a master's degree in primatology at the National University of Colombia in Bogotá. Meanwhile, he has been tramping across the ranch-flattened landscape of Caquetá to complete a census of the cat-sized monkey. But now, he says, "it's very painful" to realize that the animal is in trouble as its habitat shrinks.

Biologist Martin Moynihan was the first to suspect the presence of an unidentified species in 1976, when he saw a titi monkey lacking the typical white face stripe. Moynihan hypothesized that it was a new species or subspecies associated with swampy, humid terrain. Thomas Defler, Colombia's leading primatologist and García's academic adviser, wanted to follow up but says that "it was impossible" to venture into Caquetá. Then García arrived at the university. "Javier was willing to go and enjoyed the reputation of his father," a well-regarded local veterinarian, Defler says.

García took *Science* on a drive along 40 kilometers of highway and dirt roads, and finally a short trek by foot to visit a group of monkeys. Before setting out, García stocked up on T-shirts emblazoned with the monkey's face, miniature field guides to hand out as gifts, and a camera preloaded with pictures of animals. All of these are safety precautions. "When you start talking about monkeys in a zone with problems of armed conflict, people think you are making it up," García says.

This part of Caquetá, once Amazonian jungle, has been almost completely deforested. García found that small groups of the red-bearded titi survive in forest fragments with as few as 40 to 50 trees. Using satellite images, he and Defler estimated that in some municipalities of the region less than 2% of the original jungle cover remains. The monkey is now listed as critically endangered, one of 14 monkeys on an international list of critically endangered primates in the Americas.

Last year, Caquetá's local government directed Pacific Rubiales Energy, an oil company, to fund a \$220,000 ecological study of the monkey. The project, one of Colombia's largest conservation efforts directed at a single animal, is being led by Defler, National University geneticist Marta Bueno, and colleagues. Since the discovery, García has started a conservation group called Herencia Nacional, "national heritage," that teaches schoolchildren about the red-bearded titi. This year, he saved up to buy a truck onto which he painted the monkey's profile.

But he has grand ambitions. He hopes that the Colombian government will mandate reserves, reforestation of river banks, maybe even a carbon-trading plan. Perhaps, he says, his monkey could be the charismatic species that leads to a "change in the whole economic model that governs the landscape."

In the field. Javier García in a stand of trees that is home to the red-bearded titi monkey (below), a new and endangered species he described in 2010.



But García has returned several times to a patch of trees where his monkeys lived, only to find it burned away and the monkeys gone. "What did they do? Did they migrate?" García wonders.

With his T-shirts, guidebooks, and big smile, García is becoming a familiar presence around Caquetá's schoolrooms and vestigial forests. "I am really finding myself" as a conservationist, he says. "But I don't know if I will save this monkey."

—A. R.

no escape as temperature rises. They can only migrate upwards, until they run out of mountain. Stuart Pimm, a conservationist at Duke University, in Durham, North Carolina, warns that Colombia could be hit with a record rate of extinctions.

Return to the field

Armed groups still hold sway in many parts of Colombia, including some of the most biologically interesting areas. The lowlands of Cauca on the Pacific Ocean, for example, are risky. With 13 meters of rainfall a year, “it’s one of the wettest places on earth,” Lynch says. “The number of frogs is astonishing. ... But it’s pure narcotraffickers, and you’d be a fool to go. So it’s one of the blank spots on my map.”

Biologists are surging back elsewhere. The payoff in the Sierra Nevada de Santa Marta on the Caribbean coast, for example, has been swift. “We have gone from just dreaming of getting into these areas to having relatively good samples,” says J. Van Remsen, an ornithologist at Louisiana State University in Baton Rouge who maintains the South American checklist of bird species. Among Remsen’s finds, made near Bogotá, was *Grallaria kaestneri*, a bird last spotted in the 1990s when the initial, or type, specimen was collected. This time, Remsen says, “we also got recordings of vocalizations and a DNA sample.”

Pedraza was among those who decided the time was right to return in 2010, when she loaded up mules for an expedition into Las Orquídeas National Park, all but unvisited by international researchers since the 1990s. Her four expeditions since then have been bonanzas, she says, each yielding “four or five new species” in the group of tropical blueberry she specializes in. “This is what you get for going for a few days.”

Others echo Pedraza’s excitement. “We are discovering lots of new [bird] species” many of them not even rare, says Andrés Cuervo, an ornithologist who works with Remsen at Louisiana State University. With four Colombian colleagues last year, he described a new type of wren in the northwest of the country. Like Pedraza, he complains of red tape, including “an unbelievable bureaucracy” that is especially meticulous about permits for collecting specimens and sampling DNA, although two new decrees issued in June are expected to cut through the paperwork. As a result, he says, even though Colombia is a bird paradise, it is lagging behind its neighbors Ecuador and Venezuela in cataloging its species.



Race with development

Biologists realize that the civil conflict brought a fortuitous—and likely temporary—benefit. Even though there is growing deforestation in Colombia, the war kept many areas isolated and also led to a wide regrowth of secondary forests on land that farmers had abandoned in their retreat to the cities. This provided moisture for frogs and habitat for migratory birds. “If you leave the highway you can see the fields growing back,” in many regions, says Etter, the forest expert.

“To go to a habitat and find [that] the dominant tree has no name—well, that is pretty intense.”

—DOUGLAS DALY,
NEW YORK BOTANICAL GARDEN

With researchers at the University of Puerto Rico, Río Piedras, Ana Sánchez-Cuervo and T. Mitchell Aide, Etter published a satellite study last year reporting that between 2001 and 2010, while its neighbors lost forest, Colombia actually gained 17,000 square kilometers of woody vegetation. Although Etter thinks the evidence points toward the conclusion that war helped nature, he warns that this isn’t certain. “We don’t know what this coun-

Changing environment. A 2012 study reported on a decade’s changes in forest cover in and around Colombia.

try would have been like without the violence,” he says. Etter says that the finding has been controversial, because people don’t want to believe that any good came of the conflict.

As fighting ebbs, road builders, miners, and ranchers are racing into many of the same regions that biologists are exploring. For instance, Pedraza says that on her most recent trip, even in the most inaccessible corners, her team came across many families mining gold, part of a wider illegal mining boom in Antioquia, where the presence of more than 15,000 artisanal mines have given its towns the highest level of mercury pollution in the world.

The national government is encouraging development with tax breaks for palm oil plantations and biofuels. Foreign investments in Colombia’s petroleum sector leapt 20-fold in 2011 over the level a decade ago, to more than \$9 billion. Mining companies looking for coal and gold account for \$4 billion more invested per year.

Colombia has a large network of national parks and strong, if sporadically enforced, environmental laws, conservationists say. “It’s a world leader on paper,” says Andrew Jarvis, head of policy analysis at the International Center for Tropical Agriculture in Cali, Colombia. But, he adds, “if you look at the budget of the national parks system—it’s quite pathetic.” Colombia’s parks agency has \$30 million to protect 57 parks covering 127,000 square kilometers.

Conservationists have been trying to inject biodiversity into the debate over Colombia’s priorities. In March 2012, for instance, Conservation International awarded President Juan Manuel Santos its “Global Conservation Hero” award. Critics jeered that it was undeserved, as they claim Santos’s record isn’t very strong. But the conservation group said that the president was sending the right “signals.” A year earlier, the government responded to severe floods by citing climate change and soil erosion as national priorities. Baptiste, the Humboldt director, says that makes her optimistic. She says, “People are now talking about biodiversity as part of national security.”

—ANTONIO REGALADO

Antonio Regalado is a writer in Boston.



Coca Science Seeks An Answer in Kilos

HACIENDA LOS PIJAOS, COLOMBIA—

The noisy prop plane rumbles over the Andean highlands, crosses the muddy scrawl of the Rio Magdalena, and finally touches down on a grass runway protected by a steep ring of hills. This is the Pijaos ranch, home to the Experimental Coca Cultivation Project, the world's only coca research station. The Colombian and U.S. scientists who work on this police base have two main goals—to perfect ways to kill the coca plant and to estimate exactly how much cocaine is produced in Colombia. Their study of varieties, yields, and growing conditions supports the U.S. State Department's efforts to produce a single weighty number each year, an estimate of global cocaine output.

Millions of dollars in U.S. military anti-narcotics aid rest on this calculation, including support for Plan Colombia, which took the war on drugs into Colombia's coca fields in 1999. In July, the estimate of Colombia's potential output of pure cocaine stood at 175 metric tons per year, down 8% over the previous year, according to the United States. Colombia, once the world's largest cocaine producer by far, has fallen behind Peru.

The Pijaos researchers work in obscurity. Their target is an enigma, too: In 2012, only four articles were published on the basic biology of the coca plant. Charles Helling, a retired U.S. Department of Agriculture scientist now in Beltsville, Maryland, says that when Pijaos was picked as the site for joint Colombian-American coca research in 2006, the literature still mostly consisted of 19th century ethnobotanical studies and reports from the Coca-Cola Company, which included cocaine in its original drink recipe. "It's basically an illicit plant. There is almost no one else doing research with it," Helling says.

The Pijaos staff "replicates what the

campesinos are doing," says Ingrid Simon Calvo, an environmental engineer and scientific adviser to the U.S. State Department's Narcotics Affairs Section. "If they use more fertilizers, then we do. If they add urea, we add it ... and see if it increases yield." Since arriving at Pijaos in 2009, Simon Calvo has become familiar with a combination of soil and growth hormones that yield the most cocaine. "But we wouldn't publish it," she says.

Rows of coca plants are laid out alongside yucca, banana, and corn. They're used to calibrate multispectral cameras on U.S. Drug

Coca is "basically an illicit plant. There is almost no one else doing research with it."

—CHARLES HELLING
USDA, RETIRED

Enforcement Agency airplanes that detect coca plants by their light signature. Satellite images, studied by a branch of the CIA that once tracked Soviet grain harvests, are also used to monitor cultivation. But estimates remain slippery. One reason: Farmers have begun to grow coca on smaller or more irregular plots or under trees in an effort to confuse image interpreters.

Last summer, the United Nations produced an accounting that showed a rise in Colombia's potential cocaine production. The White House responded by putting out a press release restating its own figure, which that year had shown a steep fall of 25%. James Story, director of the Narcotics Affairs Section of the U.S. Embassy in Bogotá, says what's important is that both the U.S. and U.N. figures show a slow decadelong decline. "We think the science definitely gives us the trend, not an exact figure."

The Pijaos lab plays a little-known, but criti-

Illegal crop. U.S. environmental engineer Ingrid Simon Calvo among coca plants.

cal part in Colombia's effort to combat guerrillas, because cocaine profits finance 80% of their activity, Story says.

One line of research studies farmers' attempts to counter Plan Colombia. During the last decade, drug syndicates began bringing in tankers to spray crops with sugar water, believing it blocked the herbicide. "We needed to find out if that was just an old wives' tale," Helling recalls. Although washing does reduce the effectiveness of the herbicide, Helling found that sugar didn't have any additional effect. "So you'd kind of encourage them to do that; it's a waste of time."

"Science underpins every effort" at Pijaos, Helling says. But the work has not touched on one hotly debated aspect of Plan Colombia: whether its use of herbicides could harm people. Colombian police planes annually spray about 100,000 hectares with Monsanto's Roundup (glyphosate), which blocks plant growth. It is applied at rates that bump up against limits recommended by the U.S. Environmental Protection Agency. Many fear that the herbicide could harm farmers.

But Helling says his studies found that a lower rate of application was significantly less effective. As a result, leaders of Plan Colombia have resisted calls to cut back. "We know there is no health impact of glyphosate," says a U.S. embassy official in Bogotá.

Meanwhile, the cat-and-mouse game with growers continues. Simon Calvo says this year Pijaos researchers were using their plantations to study whether more frequent applications of lower herbicide doses might actually work better at killing the plant. The goal, as always, is to use science to "break the cycle" of cocaine production.

—A. R.



Hairy situation. Sausage-shaped *B. pertussis* lodges into cilia in the respiratory tract.

PUBLIC HEALTH

The Pertussis Paradox

The introduction of a safer vaccine has inadvertently led to a frightening spike in the deadly disease commonly known as whooping cough

When California reported more than 9000 cases of whooping cough in 2010, public health alarm bells rang far and wide. A childhood disease vanquished decades ago by a vaccine was resurgent. Major outbreaks of the disease, properly known as pertussis, soon surfaced in several other states, including Minnesota, Washington, and North Carolina. Something was wrong, very wrong.

Kathryn Edwards, a vaccinologist at Vanderbilt University in Nashville, and many of her colleagues realized that the safer pertussis vaccines they helped usher in in the 1990s had come at a steep cost: They do not create immune protection as long-lasting as the vaccine they replaced. “It’s humbling and kind of depressing,” says Edwards, whose own daughter had suffered serious side effects from the old-fashioned vaccine. “I spent so much of my time and my life working on this. ... We were so excited that we had the answer, and now it isn’t really the answer.”

The old shot had cut the number of annual cases of whooping cough in the United States to a low of just over a thousand in 1976. But as the Centers for Disease Control and Prevention (CDC) in Atlanta prepares its final tally of U.S. cases in 2012, the number is closing in on 50,000, the highest since 1955, with at least 18 deaths and hundreds of hospitalized infants. Once-rare outbreaks

are also common among vaccinated children across Europe, Australia, and Japan.

The old whooping cough vaccine, known as DTP, contained killed whole pertussis bacteria—*Bordetella pertussis*—as well as detoxified diphtheria and tetanus particles to protect against those diseases. In the newer vaccines, the pertussis component includes only purified pieces of that organism. Intensive studies are under way to try to understand why the safer “acellular” vaccines, dubbed DTaP, don’t protect for as long. Already, the studies have uncovered features of both the bacteria and the immune response to it that may be to blame. Other efforts are focused on salvaging the long-term efficacy of the old vaccine without restoring its dangers. But vaccine researchers know that solving the problem will not be easy.

Less is less

The original DTP vaccine, introduced in the 1940s, has been administered to children billions of times. But it frequently caused high fevers and seizures, which rekindled an anti-vaccine movement that had been quiet for half a century. In the 1980s, parents who blamed DTP for harming their children successfully sued manufacturers, leading many vaccine-makers to leave the market, although studies showed that permanent brain damage suppos-

edly linked to the product was extremely rare and perhaps never directly caused by the vaccine. “People used to get up at scientific meetings and shout, ‘You’re killing our babies!’” recalls Alison Weiss, a microbiologist at the University of Cincinnati in Ohio.

The actual side effects were soon linked to a powerful immune stimulant called endotoxin, contained in the cell membrane of the pertussis bacteria. This substance was removed from all the DTaP vaccines, which replaced DTP in the United States and other wealthier nations in the late 1990s.

The new vaccines seemed just as effective, without the side effects. “Our nurses did home visits and administered randomized shots. They could tell with 100% certainty who got whole-cell and who got DTaP,” Edwards says. “It was pretty remarkable—they were much less reactive. And they made antibody responses that were comparable or even higher than the whole-cell vaccine.” Today, five doses are given to kids between the ages of 2 months and 5 years, and a booster shot (with a slightly reduced dose) is administered around age 12.

The mounting bad news about pertussis outbreaks has caused great consternation among health officials, who are acutely sensitive to public distrust of vaccines—in no small part because of the old DTP’s problems.

Bruce Gellin, director of the National Vaccine Program Office at the U.S. Department of Health and Human Services, emphasizes the irony. “The DTP shot was the origin of the modern antivaccine movement, which led to a whole cascade of events, including a new vaccine that was less reactogenic—but as it turns out, at a cost,” Gellin says. Adds epidemiologist Thomas Clark of CDC’s Meningitis and Vaccine Preventable Diseases Branch: “We have acellular vaccines because people doubted the safety of whole-cell vaccines. We don’t want them to doubt the effectiveness of our pertussis vaccines.”

It took several years after DTaP came to market before its limitations became clear. The reason: The immunity that it generates wanes slowly, as Clark’s group at CDC showed in *The Journal of the American Medical Association* last November. They found that the acellular vaccines were solidly effective in the first year. But protection steadily declined over 5 years—as other studies have also shown. They revealed that children who received even a single dose of whole-cell vaccine were more than twice as likely to remain disease-free during an outbreak as those who

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received only acellular vaccines when they were infants. “These studies say the vaccine is the problem,” Weiss says.

Untangling why has been difficult, because researchers don’t fully understand how pertussis vaccines work. Even with the whole-cell pertussis shot, the bacterium often infected adults, blood sera studies have shown, but they usually did not get sick or did not realize that their coughs were due to pertussis. “It’s amazing how little we understand about pertussis and our immune response to it,” says microbiologist Tod Merkel, who heads the respiratory and special pathogens lab at the Food and Drug Administration (FDA) in Bethesda, Maryland.

The whole-cell shot contained more than a dozen different antigens, the particles that stimulate the creation of antibodies. Acellular vaccines in use around the world today contain between one and four of the antigens, including an important substance called pertussis toxin. The two vaccines now used in the United States also contain the pertussis surface proteins filamentous hemagglutinin antigen (FHA) and pertactin.

The acellular vaccines generate high levels of antibodies to these proteins—but not all of those antibodies seem to be crucial for immunity to the disease. In trials held in Sweden and Germany, for example, whole-cell vaccines proved effective at preventing disease even while producing lower antibody levels to pertussis toxin and FHA.

Pertussis “yanks the chains of the immune system,” says the University of Cincinnati’s Weiss. “It directs the immune system in the wrong way.” FHA, for example, stimulates production of interleukin-10, a chemical that stimulates antibody production but suppresses a healthy response to bacterial infections.

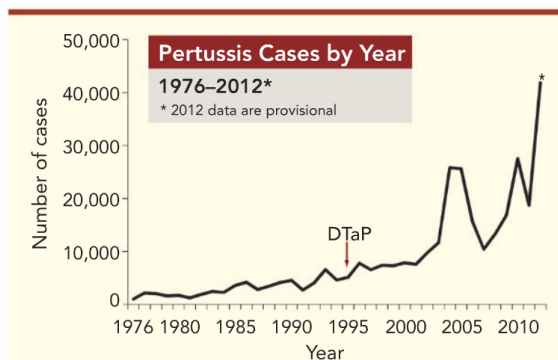
Endotoxin, in contrast, appears to be key to an effective vaccine. Although endotoxin triggers the high fevers and other adverse reactions caused by the whole-cell shot, it also sparks a hearty “innate” immune response. Unlike the adaptive immune system that produces antibodies against specific invaders, the more primitive innate immune system mounts attacks against, say, all Gram-negative bacteria like pertussis. So this nastier part of the bug may account for much of the old DTP vaccine’s longer-lasting effects.

Some studies suggest that *B. pertussis* is exacerbating a bad situation by mutating around DTaP, foiling the vaccine even during the period it stimulates a robust response. New pertussis strains recently found in the United States, France, and Australia, for example, lack pertactin, a key vaccine component. In a particularly concerning finding reported by

Clark’s CDC group in the 7 February issue of *The New England Journal of Medicine*, it found pertactin-negative strains in 11 of 12 infants hospitalized during a recent outbreak in Philadelphia, Pennsylvania. “You don’t find pertactinless isolates in our historic collection,” Clark notes.

DTaP 2.0

For now, public health officials are trying to deploy the existing vaccine more effectively. Because pertussis presents the most profound risk to unimmunized infants, CDC’s Advisory Committee on Immunization Practices (ACIP) last year recommended that pregnant women receive a booster version of DTaP. The hope is that the booster will help protect the babies for at least their first year by preventing infection in the mothers. Maternal antibodies may also pass through the placenta and breast milk and directly protect the baby, too. Unpublished British data show that an intensive maternal vaccination program begun there last October has protected infants well.



Reversal of fortune. U.S. pertussis cases have steadily climbed in the DTaP era.

But the recent outbreaks show that older children need longer-lasting immunity. A proposal to give adolescents a second booster—for a total of seven pertussis shots between birth and age 16—was tabled at the June meeting of ACIP because of a negative cost/benefit assessment. The most attractive solution is clearly an improved DTaP.

“We have to go back to research on pertussis, which has not been a priority in the recent past because we thought the problem was more or less solved,” says Stanley Plotkin, a renowned vaccinologist who consults with Sanofi Pasteur, a major producer of acellular vaccines. Although the number of labs working on pertussis has shrunk, a core of experienced researchers remains, and they have better tools than they did in the 1990s, including a new animal model. Merkel and his colleagues last year showed that the baboon

provides a far more accurate reflection of human immunity to pertussis—and a better testbed for vaccines—than the macaque monkeys in which earlier work was done.

Academic researchers and industry are pursuing a variety of approaches. New ingredients might include adenylate cyclase toxin, a protein that helps *B. pertussis* establish an infection. Novartis, of Basel, Switzerland, is looking into the reintroduction of a vaccine containing a genetically modified pertussis toxin. The vaccine performed well in trials in the 1990s, but was never licensed in the United States or most of Europe. Other researchers are eyeing the immune system stimulator, or adjuvant, in the current vaccine. It uses alum; perhaps a newer adjuvant could breathe life into the cell-free vaccine.

Some researchers want to refurbish the whole-cell vaccine so it packs a punch without causing harm. Microbiologist Camille Loch and his colleagues at INSERM, the French biomedical research agency, have developed a live pertussis vaccine in which three of the pertussis toxins have been genetically deactivated or removed. Sprayed into the nose, the vaccine protected mice well and produced a healthier immune response than acellular vaccines. It has so far been safely tested in a small number of adults. James Cherry, an infectious disease specialist at the University of California, Los Angeles, with colleagues Rachel Fernandez of the University of British Columbia, Vancouver, and Peter Sebo of the Academy of Sciences of the Czech Republic in Prague, are working on a version

of the killed whole-cell vaccine that contains genetically detoxified endotoxin.

Formulating a new vaccine and getting it licensed for use in infants presents sobering challenges, however. Changing pertussis ingredients could alter the effectiveness of the tetanus and diphtheria components in the DTaP shot—which in some places also contains hepatitis B, inactivated polio, and *Haemophilus influenza* type b antigens. All told, bringing a new vaccine to market could cost several years and hundreds of millions of dollars. Companies that profit from current pertussis vaccines may balk at the investment.

But until government and industry commit to the effort, CDC’s Clark and other epidemiologists suspect that pertussis cases in countries that use DTaP will continue to climb.

—ARTHUR ALLEN

Arthur Allen is a writer in Washington, D.C.

LETTERS

edited by Jennifer Sills

National Park Service Needs Proactive Strategy

THE RECENT DECISION BY THE NATIONAL PARK SERVICE (NPS) TO CONSIDER AUGMENTING THE beleaguered wolf population on Michigan's Isle Royale, and by doing so influence national policy, is a step in the right direction ("Are Isle Royale's wolves chasing extinction?," C. Mlot, News Focus, 24 May, p. 919). The NPS's mission—to preserve "unimpaired the natural and cultural resources and values of the national park system for...future generations" (1)—has historically been an apt guiding principle, but it may need a revision to reflect today's realities. The recent plight of another rare canid, the island fox, touted by NPS personnel as a case study in endangered species recovery (2), is another case in point.

In the 1990s, the introduction of feral pigs to Santa Cruz Island, California, drew their predator, golden eagles, to the island. In addition to the pigs, the eagles preyed on island foxes, caus-

ing the native population to decline precipitously (3, 4). After Channel Islands National Park was informed of this conservation conundrum (5), it took more than 3 years to confirm that a similar situation was occurring on nearby San Miguel Island and another year before a comprehensive recovery program was initiated (2, 6). During this brief time, fox populations on San Miguel and nearby Santa Rosa Island declined to a mere 15 individuals each (2). More than a decade later, and at considerable expense, the fox populations have recovered. However, the severe bottleneck has markedly reduced genetic variation in these

populations (7), compromising the foxes' future evolutionary potential. This weakness could have been avoided had a streamlined decision process been in place to quickly thwart the threats and avert the declines (8).

A progressive revision to the mission of the NPS, or at least its implementation, may be prudent in today's perilous times. The revised mission should be unencumbered by government bureaucracy and should rapidly implement strategies to preserve biodiversity. Augmenting populations before their extirpation or to bolster genetic diversity is exactly the type of strategy that the NPS should consider—such action could help the Isle Royale wolf and the island fox as well.

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Science Teacher Training in China

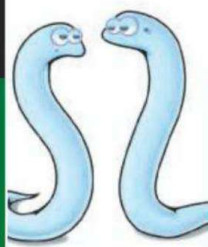
THE MOST RECENT DRAFT OF THE U.S. "NEXT generation science standards" promoted a practice-oriented approach to inquiry-based science learning (1), guided by the 2011 U.S. National Research Council framework for K-12 science education (2). The key question is whether science teachers are able to implement practice-oriented, inquiry-based learning. Training is crucial to ensure the quality of teaching described in the "Next generation science standards" report. China faces similar challenges regarding how to offer effective training.

The Ministry of Education of China began the National Teacher Training Project (NTTP) in 2010 (3) and has invested US\$89.6 million every year to provide professional development opportunities for teachers, especially training in inquiry-based learning. The NTTP cultivates lead teachers, who in turn train local teachers. Approximately 68,700 middle school science teachers have taken the 15-day training to be lead teachers since 2010 (3). Lead teachers have provided training courses of 10 to 15 days to about 100,000 middle school science teachers (3).

An effective science teacher training program, in China as well as in the United States, should engage scientists, education psychologists, and excellent middle school science teachers to provide trainees with research-oriented training. My experiences suggest that the NTTP program is meeting these goals. I have designed and led the lead science teacher training program of the NTTP in Soochow University for 4 years. We required the trainees to study science history, research the classic experiments underlying middle school science curriculum, identify problems in science and science teaching, research positive and negative cases of science instruction, and provide inquiry-based science



Island fox.



RNA on the outside

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Evolution of monogamy

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lessons in our partner middle schools and report on the experience. We hope that such thorough training will fundamentally change science education in China.

XIAOYONG MU

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Aquarium Industry Threatens Biodiversity

THE AQUARIUM INDUSTRY IS A MAJOR SOURCE of aquatic invasions, especially in terms of the number of species and potential sites of species release (1, 2). The introduction of species to foreign ecosystems occurs through intentional release of adults from aquaria into the environment, accidental release from breeding facilities (common in areas where the captive stocks are raised in outdoor farms), and unnoticed release of juveniles in discarded water (1). Regulatory and legal mechanisms that rely on accurate species-specific data are critical to preventing biological invasions and mitigating the consequences of those that occur (3).

Examples of regulation include Section 75 of Victoria, Australia's Fisheries Act of 1995 (4), and chapter 68A-23, Section 008, of Florida's Administrative Code (5), each of which declares more than 30 nonnative aquarium fish species illegal to import. Unfortunately, many developing countries lack such specific regulation, allowing the aquarium industry to introduce nonnative species into the environment. For example, in Brazil, ornamental fish farmers release commercially important families such as Cyprinidae and Poeciliidae into the creeks and later collect and sell the offspring (6).

Recently, Brazil has recorded ecological problems caused by nonnative aquarium fishes (7). In 2008, the Brazilian Institute of

Environment and Renewable Natural Resources (IBAMA) tried to reduce intentional and accidental invasions stemming from the aquarium industry by prohibiting the sale of 6 marine and 16 freshwater fishes to aquarium hobbyists (8, 9). However, since 2008, many illegal species, such as the lemon-peel angelfish (*Centropyge flavissima*) and the giant snakehead (*Channa micropeltes*), continue to be sold by the aquarium stores throughout Brazil (10, 11). The clear ineffectiveness of these bans is due to poor inspection by authorities, and specifically by the inability of inspectors and agents to identify the difference between illegal and legal species.

Modifying human behavior of all engaged in aquarium industry is the key to preventing invasions (1, 12). Interpol must monitor foreign exporters; agencies controlling borders need more fish specialists to properly identify species imported by wholesalers; and IBAMA officials should routinely inspect wholesalers and retailers (13). We must also raise awareness about the prohibitions regarding nonnative species among aquarium hobbyists, who are an important source of invasive propagules through aquarium dumping. Brazil is home to substantial biodiversity (14), and the persistent availability of prohibited nonnative fishes through the aquarium industry poses a dangerous threat to its precious environmental heritage.

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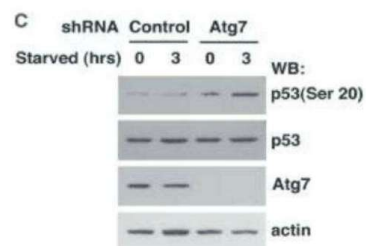
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CORRECTIONS AND CLARIFICATIONS

Reports: "Atg7 modulates p53 activity to regulate cell cycle and survival during metabolic stress" by I. H. Lee et al. (13 April 2012, p. 225). During figure preparation, duplications were introduced in Fig. 1 and Fig. 3. In Fig. 1F, the panel labeled actin was identical to the panel labeled p27. The p27 blot was used as a placeholder during preparation of Fig. 1F and was not replaced in the final figure. The actin panels in Fig. 3C and Fig. 3G were identical; in this case, the incorrect loading control was used in preparation of Fig. 3C. Corrected versions of Fig. 1F and Fig. 3C are shown here. The errors do not affect the conclusions of the paper. The figures have been corrected in the HTML and PDF versions online.



Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

CONSERVATION BIOLOGY

Is Embracing Change Our Best Bet?

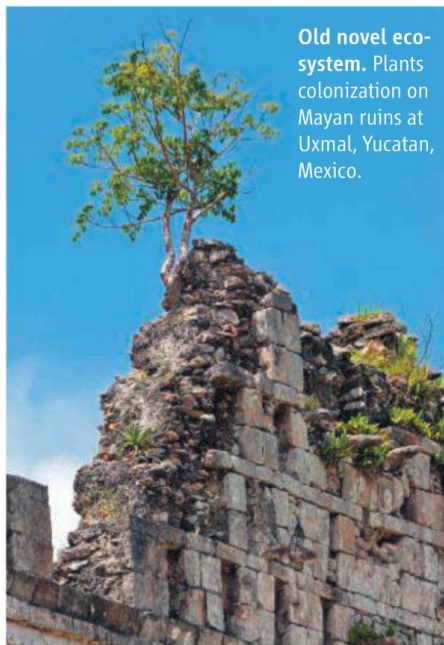
David Moreno Mateos

Restoration ecology and conservation biology are both under pressure to adapt to accelerated anthropogenic global change. Pristine areas free from human influence no longer exist and, arguably, have not for thousands of years (1). Major land-cover transformations for agriculture affected vast territories more than 3000 years ago (2). Large mammal extinctions in the late Pleistocene (circa 12,000 years ago) were related to human expansion (3). And relocation of now-widespread naturalized species was already happening 4230 years ago, when domestic dogs (dingos) were introduced into Australia by way of southeast Asia (4). Thus, human-sculpted landscapes are what we have been mostly managing for millennia. Because the rate of alteration has dramatically increased over the past 200 years, those ancient localized impacts now affect most of the world. Additionally, other indirect impacts act at a planetary scale—e.g., increased carbon dioxide concentration and nitrogen deposition.

Since the late 19th century, conservationists have sought to preserve habitats, populations, and species from further degradation or reduction. Since the 1980s, practitioners of ecological restoration have tried to assist damaged ecosystems with recovering from human impacts (5). In the face of massive anthropogenic change, ecologists and ecosystem managers frequently struggle to study and to manage such amounts of change, and neither conservation biology nor restoration ecology,

in their current forms, provides sufficient conceptual basis or all the tools necessary to move forward (6). In *Novel Ecosystems*, editors Richard Hobbs, Eric Higgs, and Carol Hall and their contribu-

tors present theory and case studies to support an alternative approach to addressing human-induced change in ecosystems around the world. Anthropogenic change is not inherently bad, they argue. It should be accepted and managed in ways that increase ecosystem



Old novel ecosystem. Plants colonization on Mayan ruins at Uxmal, Yucatan, Mexico.

functionality and, in turn, increase ecosystem services provided to humans. The editors define the titular “novel ecosystems,” introduced in (7), as “systems that differ in composition and/or function from present and past systems as a consequence of changing species distributions, environmental alteration through climate and land use change and shifting values about nature and ecosystems.”

The volume includes well-documented examples that highlight how nonnative species appear to contribute, at least over short time scales, to the conservation of native endangered species. At present, both the endangered Rodrigues fody (*Foudia flavicans*) on Rodrigues Island and the Carnaby’s cockatoo (*Calyptorhynchus latirostris*) in southwestern Australia rely on seeds of invasive trees. Introduced species assemblages may also play important functional roles. On Rodrigues Island, the Aldabra giant tortoise (*Aldabrachelys gigantea*) imported from the Seychelles feeds on native and nonnative plants, replacing the recently extinct endemic tortoises and bringing back herbivory. Several contributors claim that after the “original” (i.e., roughly pre-Anthropocene) species composition is lost, functionality, at least, may be recovered through nonnative species. These authors suggest that ecologists and managers reassess their value-laden perceptions of heavily altered, degraded, or invaded

ecosystems and potentially embrace such systems as a “new normal” that may help provide valued services to humans.

The authors of the chapter on a conceptual framework hold that novel ecosystems differ from other ecosystems because human activities have pushed them across ecological thresholds to a new state where they “do not depend on human intervention for their maintenance.” Threshold crossings can be provoked by anthropogenic environmental changes and by socioeconomic or political circumstances. The authors identify as “hybrid” those ecosystems that combine characteristics of both novel and historical systems because they have suffered substantial alteration but have not yet crossed a putative ecological threshold. They propose that these thresholds provide tools for ecosystem management. For hybrid ecosystems, managers should work to dampen or reverse drivers causing the shift. They may be able to restore, at least partially, historical species configurations. However, once the threshold is crossed, the historical biodiversity and functionality of the system are lost. In which case, managers should focus on maximizing functionality in the novel ecosystem.

Unfortunately, threshold crossings are only occasionally reported in the scientific literature. Because they are driven by a variety of causes and may take decades or centuries to exhibit detectable symptoms, they are difficult to recognize. So it seems unlikely that highly uncertain, trial and error-based protocols for detecting threshold crossings will soon be embraced by already-burdened ecosystem managers. At present, these thresholds look more like theoretical constructs than practical management tools. However, the volume highlights the urgent need to further develop their potential.

The editors and contributors acknowledge that change “is an inherent property of ecosystems” and that today all ecosystems are directly or indirectly influenced by human activities. Thus, the term novel ecosystem essentially refers to any ecosystem, which suggests that we can dispense with its use. Also, because nothing is permanently new and, according to the authors, novel ecosystems perpetuate themselves, the term is conceptually contradictory. Commenting on the scope of the concept, Emma Marris, Joseph Mascaro, and Erle Ellis justify its relevance because of its practical value for ecosystem management as a “propaganda tool”—a means “to rebrand lands currently described by ecologists as ‘degraded.’” However, widespread acceptance of this simplified perspective of the concept may have two major unde-

Novel Ecosystems

Intervening in the New Ecological World Order

by Richard J. Hobbs, Eric S. Higgs, and Carol M. Hall, Eds.

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sired consequences. There may be perverse outcomes, similar to the results from some national mitigation policies under which further degradation of “undisturbed” wetlands is often justified by ecosystem restoration that usually delivers a less diverse and less functional state (8). And the term might lead to imprudent management, such as the relaxation of early detection and effective controls on biological invasions, with unpredictable outcomes.

Conservation biology and restoration ecology are changing rapidly, providing new conceptual foundations and tools for preserv-

ing or restoring biodiversity and ecosystem functionality, both of which are now considered global priorities (9). The disciplines need more time to mature and produce optimal solutions to our growing concerns. Although the authors’ new terminology does not seem a step forward, *Novel Ecosystems* provides relevant and stimulating ideas for discussion and integration into conservation and restoration methods, strategies, and goals.

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NEUROSCIENCE

A Lifetime Without Memory

Nicholette Zeliadt

Henry Molaison had a poor memory; that much he knew. He could recognize family members and recall information he had learned in school, but he was unable to remember any new experiences, from the people he met to the places he visited and conversations he had. Any new information he encountered simply slipped from his mind within 30 seconds, the result of an experimental brain operation that arguably saved his life but robbed him of the ability to form long-term memories. Undoubtedly devastating to his daily life, his memory loss proved to be a priceless gift to neuroscience, facilitating a number of important discoveries that form the basis for much of what we now know about the biological underpinnings of learning and memory.

Known publicly only by his initials “H.M.” until his death at age 82 in 2008, Molaison famously underwent surgery in 1953 in an attempt to alleviate the debilitating epileptic seizures he had experienced since childhood. The procedure (which involved removing much of his hippocampus, amygdala, and surrounding structures) dramatically curtailed his seizures but left his mind forever stranded in time. In *Permanent Present Tense*, Suzanne Corkin takes readers inside the life and mind of the man behind the initials. She provides a touching yet unsentimental glimpse of her 46-year connection to this “pleasant, engaging, docile man” and his tragedy, interests, and experience of everyday life. At the same time, Corkin skillfully uses stories about his experiences and capabilities to illustrate

some of the scientific principles underlying memory. She also offers a comprehensible historical sketch of the study of memory and the burgeoning field of neuroscience—from the dubious and gruesome practice of prefrontal lobotomy to the development of powerful brain-imaging techniques.

Corkin, a behavioral neuroscientist at the Massachusetts Institute of Technology, began studying Molaison in 1962 while she was a psychology graduate student at McGill University. Corkin and other researchers devised clever cognitive tests to assess his memory and other intellectual abilities, and they subsequently used brain-imaging tools to precisely determine what structures were missing from his brain. Molaison’s case helped to reveal that something as abstract as converting a thought or experience into a memory could be localized to a discrete part of the brain—the hippocampus.



Happy to participate. Henry Molaison at a 1986 testing session at MIT.

Permanent Present Tense

The Unforgettable Life of the Amnesic Patient, H.M.

by Suzanne Corkin

Basic Books, New York, 2013. 400 pp. \$28.99, C\$32. ISBN 9780465031597. Allen Lane, London. £20. ISBN 9781846142710.

Yet to everyone’s surprise, Molaison sometimes was able to remember things, exquisitely described by Corkin as appearing “from time to time like driftwood washing up from an empty sea.” For instance, he could learn

the instructions for some of the cognitive tests that he took repeatedly and sketch the floor plan of a house that he moved into years after the onset of his amnesia. In old age, he learned to use a walker, although he had no memory of having done so. Molaison’s case aided the recognition that memory is not a single process and that some skills and information could be acquired without conscious awareness.

Molaison remained a good-natured and cooperative research participant throughout his life, his keen sense of humor ever-present. For example, when asked whether he had slept well one night during a visit to the research facility, Molaison responded, “I didn’t stay awake to find out.” He seemed to live relatively free of the stresses and anxieties of daily life, “unencumbered by recollections from the past and speculations about the future” that prevent many of us from experiencing life in the here and now. After his death, Molaison’s brain was preserved and shaved into 2401 slices for further study, and in this form, he will continue to advance our understanding of memory and the brain.

Sadly, Molaison’s condition prevented him from ever fully grasping the importance of his contributions to science and humanity. Corkin’s compelling account in *Permanent Present Tense* should help ensure that he will remain an unforgettable figure in the continuing saga of our quest to understand the workings of the mind.

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Reforming China's S&T System

Cong Cao,^{1,2*} Ning Li,^{3,4*} Xia Li,^{5*} Li Liu^{6*}

There is growing anxiety among Chinese political and scientific leaders that, despite more money, better-trained talent, and sophisticated equipment, the domestic innovation system is still underperforming (1). Chinese scientists have yet to produce breakthroughs worth a Nobel Prize. Research and business sectors have been disconnected for decades, with few research results turned into innovative technology and products. With few exceptions, Chinese enterprises depend on foreign sources for core technologies (2). We discuss interwoven roots of these problems at macro, meso, and micro levels of governance of the science and technology (S&T) system and the need for political will if they are to be overcome.

Governance Structure and Evolution

China's S&T governance is highly bureaucratized, as is that in other areas (3). At its apex is the Chinese Communist Party Central Committee (CCPCC), which leads China's S&T enterprise through the Leading Group on Science, Technology, and Education (LGSTE) at the State Council. Chaired by the premier, who is a member of the CCPCC Politburo Standing Committee—China's de facto top governing body—the LGSTE comprises heads of ministries involved in S&T, as well as the minister of finance (4). The LGSTE studies and reviews major S&T and education policies and programs and coordinates important intergovernmental activities (5).

The Ministry of Science and Technology (MOST) is an overarching agency overseeing S&T affairs, from formulation of policies, plans (*guihua*), and programs (*jihua*) to budgeting and allocation of resources for some national research and development (R&D) programs (6). The Chinese Academy of Sciences (CAS) and Chinese Academy of Engineering (CAE) provide consultation for government decision-making. The National People's Congress (NPC), China's highest

organ of state power and legislature—through its Standing Committee and the Committee on Science, Technology, Education, and Health—has authority to enact and amend S&T-related laws. The NPC monitors implementation of laws and approves budgets.

With the reform of the S&T system in 1985 (7), China's science bureaucracy started to expand its turf. The State Science and Technology Commission (SSTC) extended its role from formulation and implementation of policies at the macro level to the initiation and management of programs and projects and associated resources at the meso level. The MOST, which succeeded the SSTC in 1998, allocated and distributed roughly 14% of government fiscal S&T expenditure in 2011, further departing from its roles in making and implementing S&T policy.

Increasing involvement of ministries and industrial enterprises has diversified the nation's S&T system, with important and mixed implications for governance, coordination, and funding. In 2012, China's total R&D expenditure exceeded ¥1 trillion (U.S. \$162.9 billion), an 18% increase from 2011 (8, 9). Under the 2006 National Medium- and Long-Term Plan for the Development of Science and Technology (MLP), R&D activities funded at S&T mission-oriented agencies (e.g., ministries of agriculture, health, and industry and information technology) have increased.

Macro: Lack of Coordination

The LGSTE has not fully functioned in macro-level coordination between agencies and central and local governments. Although the LGSTE is chaired by the premier, the issues on science, innovation, and education are probably less important than others in his portfolio. Real leadership is normally held by a vice premier or state councilor, who may also have other priorities and whose power depends on position in the party and state administrative apparatus. The LGSTE is operated within a secretariat bureau under the general office of the State Council, which has many other responsibilities and priorities, with neither sufficient manpower nor incentive to coordinate.

Ad hoc in nature, the LGSTE is not involved in the budgeting process. It has never issued an official document in its own

Intergovernmental coordination, funding distribution, and performance evaluation must all be improved.

name and has failed to take actions on interruptions of the S&T system. For example, when severe acute respiratory syndrome (SARS) struck China in early 2003, lack of coordination made it difficult for researchers under different jurisdictions—civilian and military; central and local; and health, science, and technology—to respond in a timely and effective fashion (10).

When the MOST succeeded the SSTC, its coordination mission was no longer clearly spelled out. The MOST is only one ministry among many within the State Council and probably a weak one in terms of importance and clout, although it still gets the largest amount of central government S&T appropriations. Mission in S&T has been dispersed into many ministries or ministry-level organizations within the State Council, each authorized to propose and administer national R&D programs under its jurisdiction with its budget directly appropriated from the Ministry of Finance. Neither the LGSTE nor the MOST is mandated to coordinate them.

Meso: Malfunction in the Funding System

Ineffectiveness in macro-level coordination influences distribution of resources at the meso level. Normally, once a national R&D program is established and funded, the corresponding government agency issues its own guideline and handles its own (largely internal) proposal submission, review, and award process. There is no uniform, national quality-control standard, nor is there much exchange of information about projects funded across different agencies. Despite having respective research priorities (11), these national R&D programs likely overlap, under the administration of different ministries and organizations (even within the same ministry) that may set the same goals and have the same groups of scientists in mind.

Duplication is not necessarily bad, and clustering is inevitable in research and often has good results. In countries such as the United States, pluralism in funding sources is often considered a strength because it stimulates competition and diversifies priorities. Moreover, a transparent and credible review process guarantees broad input representing the scientific community and interests across government agencies, which is further over-

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seen by congressional committees (12).

In China, however, some scientists could use almost the same idea to apply for grants from different government agencies and have almost identical projects funded. Government agencies, eager to show the central government that their programs are worth the investment, are willing to fund a small number of well-established researchers with the expectation that their achievements would later be used to justify the funding and even become the sponsor's own credentials.

China's research culture gives too much competitive advantage to established researchers and those who maintain close relations (*guanxi*) with government officials, which increases research-funding inequality and concentration (13). Some scientists, mostly scientists-turned-administrators, are able to get major financial support from multiple sources, whereas other scientists, often junior and in underdeveloped regions, have difficulty securing the necessary funding to start their careers. A substantial portion of public funding at almost every ministry is funneled to favorable scientists, often well-established, through earmarks or applications received informally, rather than through rigorous and fair peer reviews.

Micro: Flawed Evaluations and Incentives

China has a weak culture of performance evaluation (14). There is an overwhelming "publish-or-perish" orientation (15, 16), especially toward publications in journals cataloged by the Science Citation Index (SCI). Although this may have contributed to growth in China's international papers, SCI publications have become an inappropriate yardstick in evaluation of research programs, institutions, and scientists. The Institute of Scientific and Technical Information of China ranks universities and institutes by the number of SCI papers. Many institutions mechanically evaluate and promote scientists according to their number of SCI publications and impact factors of the journals (17).

Doctoral students are required to have a certain number of SCI publications before being allowed to defend their dissertations; the doctoral degree could be withheld if a student fails to meet the publication criteria. The national scholarship for graduate students introduced in 2012 is tied into SCI papers at many universities and R&D institutes. Over-emphasis on the number and impact factor of publications gives rise to the phenomena of multiple first and corresponding authors and

publishing in least publishable units to meet publication criteria or get rewarded (18, 19).

Emphasis on SCI papers and impact factors in performance evaluation at the micro level informs funding decisions at the meso level. Those who are capable of producing higher SCI-impact factor papers tend to stand out in the competition. As a result, scientists are motivated to publish for the sake of publications and grants rather than finding genuine solutions to societal problems.

The evaluation culture could have negative



A guard outside the closing session of the 2013 NPC. In the background is the Great Hall of the People in Beijing.

impacts on career development. Promotion has become a rent-seeking and rent-making opportunity for a small number of persons in the review panel who have power to partly determine the destiny of candidates. At some institutions, the process is neither transparent nor fair, as evaluation regulations are not clear or not strictly followed. It may involve corruption, as the promotion could be obtained with *guanxi*, administrative or bureaucratic power, and even money. Cases involving conflicts of interest are not rare. Internal deliberations, including membership election at the CAS and CAE, which is supposed to be strictly confidential, could become public information to those who have *guanxi*. Scientists-turned-administrators are often evaluated favorably and promoted quickly.

Central and local governments and institutions have introduced programs, e.g., the Thousand Talent Program, to attract and retain talent who have foreign Ph.D.'s and research experience. Such programs have largely discriminated against domestically trained scientists. Promotion tends to give priority to those with foreign training and research experience; some positions are reserved only for them. Given the vigorosity of training and recruitment of talent at foreign institutions, it is understandable that China favors its future scientific leaders from among those with for-

ign credentials. But many returnees recruited into such programs are not necessarily high-end talent.

Worse, some returnees were able to use fabricated foreign credentials to get into such programs (20, 21). Many returnees seldom work full time in the Chinese institutions as required. Some were able to benefit multiple times from such programs, although they should no longer be treated as returnees once they have their first full-time appointments in China. The government had to initiate programs with less lucrative packages to alleviate tensions from those without foreign experience or who returned before the foreign-preferring programs were established.

Having recognized serious problems in performance evaluation, scientific and political leadership called for an evaluation system with "Chinese characteristics" (22). Nonetheless, "Chinese characteristics" should not be an excuse to ignore international norms.

Conclusions and Discussions

China's strategic objectives to become an innovation-oriented nation have been set, but insufficiency in macro-level coordination, meso-level funding, and micro-level performance evaluation reduces the effectiveness and efficiency of the S&T system. Problems at the three levels are interrelated; remedy of governance deficits at one level is unlikely to improve the overall performance.

The current LGSTE mechanism has failed in coordinating efforts at various government agencies. Given its rank within China's State Council, the MOST is unable to coordinate national S&T affairs at the macro level or to play a more important role in S&T-related policy-making. Suggestions were made, in drafting the MLP and earlier in response to the SARS crisis, to replace the MOST with a new agency akin to the U.S. Office of Science and Technology Policy (OSTP) and a national science advisor under the State Council (23).

Another solution would be to maintain the current institutional setting of the LGSTE and the MOST while creating a smaller Office of Science and Technology (OST) directly under the State Council, similar to the U.S. OSTP. Headed by a full-time National Science Advisor focusing on S&T-related issues, the office would support the LGSTE, which, in turn, becomes an organization similar to the U.S. National Science and Technology Council (NSTC, composed of government S&T leaders). In addition to the missions of the

LGSTE, the office would be responsible for overseeing national R&D programs and budgets. However, in addition to NSTC, the U.S. OSTP also staffs the President's Council of Advisors on Science and Technology, representing academic and corporate interests (12). Would China create such a council?

Distribution of resources at the meso level is problematic owing to the lack of coordination at the macro level. Maintaining information flow between ministries is critical; so is seeking accountability of those in charge of such programs. The proposed OST could play such a role. Moving the National Center for Science and Technology Evaluation from the MOST to OST would make possible independent, fair, and objective evaluation of national R&D programs, national 5-year S&T plans, and even MLP. Strengthening the NPC role in securing, negotiating, appropriating, and monitoring budgets and spending would make ministries accountable.

External reviews of China's S&T system reform in the mid-1990s and innovation policy in the early 21st century suggest that China may draw on international experience in reforming its S&T system (14, 24). Most recently, invited by the National Natural Science Foundation of China (NSFC) and the Ministry of Finance, an independent international panel reviewed funding management at the NSFC. While applauding the NSFC research-funding mechanism, the panel called on the NSFC to improve protection of confidentiality of the review process and to avoid real or perceived conflicts of interest (25).

Emphasis on SCI publication probably is a mixture of Chinese and Western, as well as socialist and capitalist, norms and practices. To improve, evaluation should follow international practice and maintain its integrity. Performance evaluation at some institutions has gradually introduced international review. For example, the CAS is in the process of having more of its institutes scrutinized by its international peers.

Immediate actions must be taken to solve problems at the micro level as well. The conflict-of-interest policy must be reinforced for both program evaluation and individual performance appraisal. Impacts of bureaucratic power and *guanxi* should be minimized. A more reasonable reward system must be

introduced that uses peer review rather than counting of publications and impact factors, to incentivize researchers to solve problems rather than just focus on publications.

These governance challenges have largely been recognized by political and scientific leadership (see the text box). Political leadership after the CCP National Congress in 2012 and the NPC in 2013 signals continuity in

RECENT STEPS TOWARD REFORM

A 2012 National Conference on Science, Technology, and Innovation led the CCPCC and State Council to propose reforms to the S&T and innovation systems (26) with the goal to further implement the MLP. In November 2012, the 18th CCP National Congress reaffirmed the guidelines set in the CCPCC and State Council document (27). In particular, the document urges that actions be taken: (i) to clearly define missions of national R&D programs, (ii) to separate entities of funding, research, and performance evaluation for the sake of checks and balances and accountability, (iii) to apply different standards to the evaluation of different types of R&D activities, and (iv) to make the reward systems more open and transparent. A new Leading Group of State Scientific and Technological Reform and Innovation System Construction was convened with representatives from 26 government agencies and headed by Liu Yandong of the CCPCC Politburo. Its mandate is to coordinate reform of China's S&T and innovation system and discuss and approve regulations (28). Liu, in charge of S&T affairs in recent years, was promoted to vice premier during the 12th NPC in early 2013. While visiting the CAS on 17 July 2013, CCP general secretary and Chinese president Xi Jinping stressed deepening reform of the S&T system and eradicating all the institutional barriers to innovation (29).

leadership of the S&T system, which is both positive and negative. Leadership is fully aware of the problems so as to know how to tackle them if it desires to; but it is also partially responsible for many of the problems and reform may not be in its best interest.

The LGSTE is supposed to lead in defining roles and responsibilities of agencies and coordinating intergovernmental relations during policy implementation. But it remains to be seen whether coordination of China's S&T could be resolved without institutional change. Operational rules, regulations, and procedures will need to be set up by appropriate government agencies. Efforts are needed to monitor implementation, get feedback, and revise rules and regulations. Challenges in governance of China's S&T system have existed for so long and the inertia to maintain status quo is so strong. It is time for leadership to show political will.

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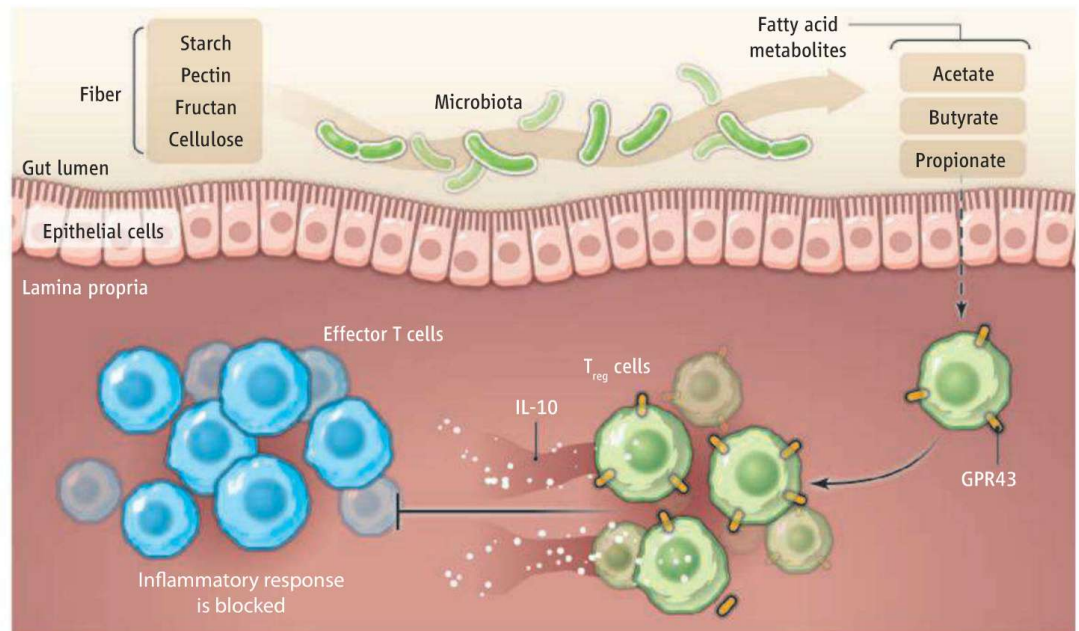
IMMUNOLOGY

Feed Your T_{reg}s More Fiber

Julia Bollrath and Fiona Powrie

The human intestine harbors up to 10^{11} bacteria per gram of intestinal content, comprising over 500 different species (1) that have coevolved with their hosts in a mutually beneficial relationship. These bacterial communities promote human health through effects on nutrition and immune system development and function, changing in distinct ways over time and in different disease states. Precisely how bacteria communicate with their hosts to promote immune function is poorly understood. On page 569 of this issue, Smith *et al.* (2) show that common bacterial metabolites—short-chain fatty acids—selectively expand regulatory T (T_{reg}) cells in the large intestine. T_{reg} cells suppress the responses of other immune cells, including those that promote inflammation. This finding provides a new link between bacterial products and a major anti-inflammatory pathway in the gut.

Intestinal homeostasis and the maintenance of gut health require an appropriate balance between immune effector and regulatory pathways. T_{reg} cells (those that express the transcription factor Foxp3) are abundant in the intestine and play a nonredundant regulatory role, particularly through their production of the anti-inflammatory cytokines transforming growth factor- β (TGF- β) and interleukin-10 (IL-10) (3). Intestinal bacteria can control this balance. For example, segmented filamentous bacteria (*Clostridium* genus) induce the accumulation of effector T cells in the small intestine of mice (4), whereas a mixture of clostridia species of the subclusters IV and XIVa prompt the expansion of colonic T_{reg} cells (5). Furthermore, *Bacteroides fragilis*, a commensal bacterium



Bacterial metabolites fight intestinal inflammation. Commensal bacteria metabolize fiber and generate short-chain fatty acids. These fatty acids are ligands for GPR43 expressed by T_{reg} cells and stimulate their expansion and immune-suppressive properties such as the production of IL-10, thereby controlling proinflammatory responses in the gut.

common in the human intestine, promotes expression of IL-10 by T_{reg} cells (6).

Smith *et al.* observed that feeding germ-free mice three short-chain fatty acids (propionate, acetate, butyrate), individually or in combination, increased the frequency and number of Foxp3 T_{reg} cells in the large intestine to an amount similar to that observed in conventionally reared animals. Different bacterial families in the large intestine can produce short-chain fatty acids as end products of the fermentation of complex carbohydrates such as dietary fiber. The majority of the expanded T_{reg} cell population expressed the transcription factor Helios, which suggests that they acquired Foxp3 expression in the thymus. This is of particular interest because bacterial-driven T_{reg} cell generation in the intestine is thought to occur locally and result in T_{reg} cells that do not express Helios. Propionate also increased the expression of Foxp3 and IL-10, but not TGF- β in colonic T_{reg} cells, indicating that short-chain fatty acids can also selectively enhance T_{reg} cell function.

Propionate showed a superior effect on T_{reg} cell expansion compared to acetate and butyrate, which may be explained by its

higher affinity (7) for G protein-coupled receptor 43 (GPR43), a receptor for short-chain fatty acids (8). GPR43 was thought to be primarily expressed by adipocytes, intestinal epithelial cells, and leukocytes (neutrophils and monocytes). However, Smith *et al.* show that intestinal T_{reg} cells also express GPR43, which is driven by the presence of bacteria. Importantly, the observed effect of short-chain fatty acids on T_{reg} cell expansion was abrogated in mice lacking GPR43. This does not appear to be a nonredundant mechanism as conventionally reared *Gpr43*-null mice did not show any deficiency in their T_{reg} cell frequency under steady-state conditions. Mechanistically, Smith *et al.* show that short-chain fatty acids are natural inhibitors of histone deacetylases (HDACs) 6 and 9, consistent with the promotion of T_{reg} cell function by HDAC inhibition (9).

Does GPR43 engagement enhance T_{reg} cell suppressive function? Incubation with short-chain fatty acids indeed increased the suppression of effector T cell proliferation in a GPR43-dependent manner. Similarly, feeding conventionally reared mice either the short-chain fatty acid mix or propionate alone enhanced the ability of intestinal T_{reg}

cells to ameliorate colitis in a mouse model of inflammatory bowel disease.

Clostridia species belonging to cluster IV and XIVa are a prominent source of acetate, butyrate, and to a lesser extent propionate in the colon (10). However, these bacteria increase the development of T_{reg} cells (those that do not express Helios) by inducing epithelial cells to produce TGF- β (10). This suggests that there are distinct pathways through which intestinal bacteria can influence intestinal T_{reg} cells. A combination of clostridia species (cluster IV and XIVa) stimulate local differentiation of T_{reg} cells, whereas administration of short-chain fatty acids leads to GPR43-dependent accumulation of thymus-derived T_{reg} cell populations. Further studies are required to determine whether GPR43 signaling in T_{reg} cells boosts proliferation or promotes survival, and to characterize the relative role of this mechanism in sustaining T_{reg} cell activity in the intestine.

How can intestinal bacterial communities be exploited to influence immune responses that benefit the host? One approach is to feed

specific “substrates” to the host that would preferentially expand beneficial bacteria—so-called prebiotics. However, the risk of increasing closely related but detrimental species through this approach cannot be ruled out. Thus, while members of the clostridia class are associated with spurring an increase in anti-inflammatory T_{reg} cells (5), an expansion of the closely related *Clostridium* cluster XI has been observed in mice sustained on a high-fat diet (11). In this case, a shift in the intestinal microbiome resulted in increased amounts of the bacterial metabolite deoxycholic acid, which in turn led to a higher incidence of liver cancer. This illustrates how closely related bacteria can have distinct effects on the host.

A different approach is to isolate beneficial bacterial species and enrich them within the existing bacterial community of the host. The isolation of 17 human clostridia species that can stimulate the expansion of T_{reg} cells in mice (10) opens up therapeutic options, but whether feeding these species will result in persistent changes in the pre-existing bacterial communities and lasting

effects on host immunity remains unclear. Perhaps the most appealing strategy is to identify the bacterial components or metabolites that affect host immunity. This would allow modification of immune cell activity without tampering with microbiota composition. Indeed, Smith *et al.* demonstrate the potential therapeutic value of understanding how bacterial components and metabolites promote human health.

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PHYSICS

Spinning the Doppler Effect

Lorenzo Marrucci

The sound from a moving object undergoes a pitch change—the Doppler effect—caused by the acoustic wave frequency increasing as the object approaches and decreasing as it moves away. A similar effect—a blue or red color shift—occurs for light from moving astronomical objects. Waves scattered off a moving object are also subject to a Doppler shift, an effect used in sonar and radar systems to deduce speed. A spinning object may also induce a Doppler effect in backscattered waves if the rotation axis is not pointing directly at the wave source and detector. The spinning object can thus be seen, at any given time, as composed of parts that are moving toward the detector and parts that are receding from it. However, if the rotation axis points toward the detector, this “piecewise” Doppler effect vanishes. This limitation can now be overcome by using “twisted” light. On page 537 of this issue, Lavery *et al.* (1)

demonstrate that a spinning object with an optically rough surface may induce a Doppler effect in light reflected parallel to the rotation axis, provided that the light carries orbital angular momentum (OAM).

Under suitable conditions, a laser beam can be associated with an electromagnetic energy flow that, while propagating forward, is also continuously turning around the beam axis. Waves of this kind have OAM parallel to the propagation direction (2), whose value per photon is given by an (arbitrarily large) integer times the reduced Planck’s constant $\hbar$ ($h/2\pi$). Note that OAM should not be confused with the “spin angular momentum” (SAM) that is associated with circular polarization and limited to $\pm\hbar$ per photon. Light waves with nonzero OAM present a helical-shaped wavefront with a well-defined center, where an optical vortex is located (see the figure). Applications of OAM include particle manipulation (3), optical nanolithography (4), high-bandwidth communication (5), and quantum information (6).

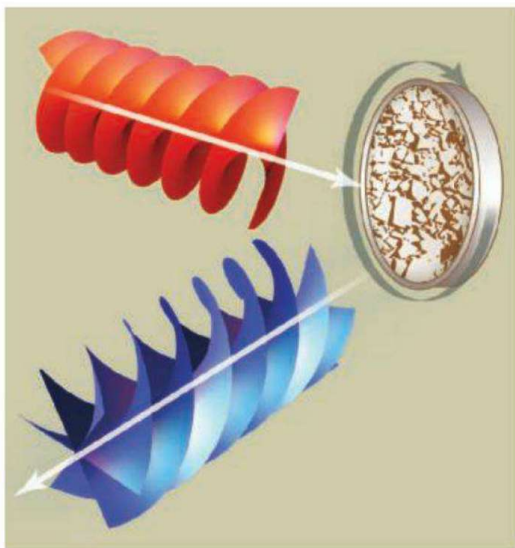
In the experiments by Lavery *et al.*, a collimated laser beam is directed at a spinning

Detection of an object’s rotation by exploiting the orbital angular momentum of light may find applications in remote sensing and astronomy.

disk, and the reflected light is detected and its frequencies analyzed. The frequency analysis is done by looking for intensity “beating” effects that reveal even tiny shifts. Normal laser beams did not show any Doppler shift, as expected. Conversely, when light with nonzero OAM was used (more precisely, with two opposite OAM components), a frequency shift proportional to the disk’s angular speed was detected, as expected for a Doppler effect, but also proportional to the optical OAM (see the figure). Because the OAM has no upper bound, the shift can be made arbitrarily large for a given disk speed.

A detailed analysis shows that the frequency shift is actually associated with a variation of OAM in the scattering process and not just with the nonzero input OAM. A perfect, specular reflection does not alter the OAM (apart from a sign change arising from the propagation reversal) and hence induces no Doppler shift. Scattering from an inhomogeneous surface (diffuse reflection) instead generates wave components carrying different values of OAM that undergo different Doppler shifts, acquiring many

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Twisted light and the Doppler effect. Light carrying orbital angular momentum (OAM), represented by its helical-structured wavefront in orange, is reflected off a spinning disk. The disk's surface roughness generates new OAM components in the scattered light. In this example, a single-helix wave with an OAM of $\hbar$ (one rotational quantum) is scattered into a triple-helix wave with an OAM of $3\hbar$. The scattered light then also acquires a Doppler frequency shift, represented here as a change of color to blue.

frequencies. However, only one frequency proportional to the input OAM will survive in the total power of the scattered light when two opposite OAM waves are used as input. This analysis indicates that a Doppler shift should occur even with ordinary “non-twisted” light as the input, if only specific nonzero OAM components are detected in the scattered light. This additional test was carried out by Lavery *et al.*, confirming the predictions.

The work of Lavery *et al.* builds on several previous results. A form of Doppler

shift arising from the transverse translational motion of a scattering inhomogeneous surface is a well-known effect, which provides the basis for the so-called laser speckle velocimetry that allows for noncontact surface-speed measurements (7). The present study can be seen as a generalization of speckle velocimetry to the rotational case. Various examples of Doppler-like effects in the interaction of light with spinning particles or molecules have been reported (8), but these effects arise from SAM scattering, not OAM. Hence, they require the particles to be anisotropic (rather than inhomogeneous) and, because SAM is bounded by $\hbar$, cannot be enhanced at will, in contrast to the case with OAM. Finally, a Doppler effect relying on OAM was demonstrated in an ad hoc setup involving transmission through spinning Dove prisms, which are truncated prisms used to invert and rotate images (9).

The rotational Doppler effect demonstrated by Lavery *et al.* could find applica-

tion in noncontact remote measurement of angular speeds. Particularly fascinating would be the detection of astronomical object rotations by filtering specific OAM components in the detected radiation (10). However, high-OAM components in the light of distant sources are expected to be strongly attenuated, so the potential in this area will require further study.

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GENETICS

Y Weigh In Again on Modern Humans

Rebecca L. Cann

The age of the most recent man or woman from whom all living humans today descended has been the subject of considerable debate. It has been suggested that the date of our last common maternal ancestor could have been three times older than that of our last common paternal ancestor. Two papers in this issue independently redate our most recent common paternal ancestor and find that there is rather little or no disparity with the age of our common maternal ancestor. On page 565, Francalacci *et al.* (1)

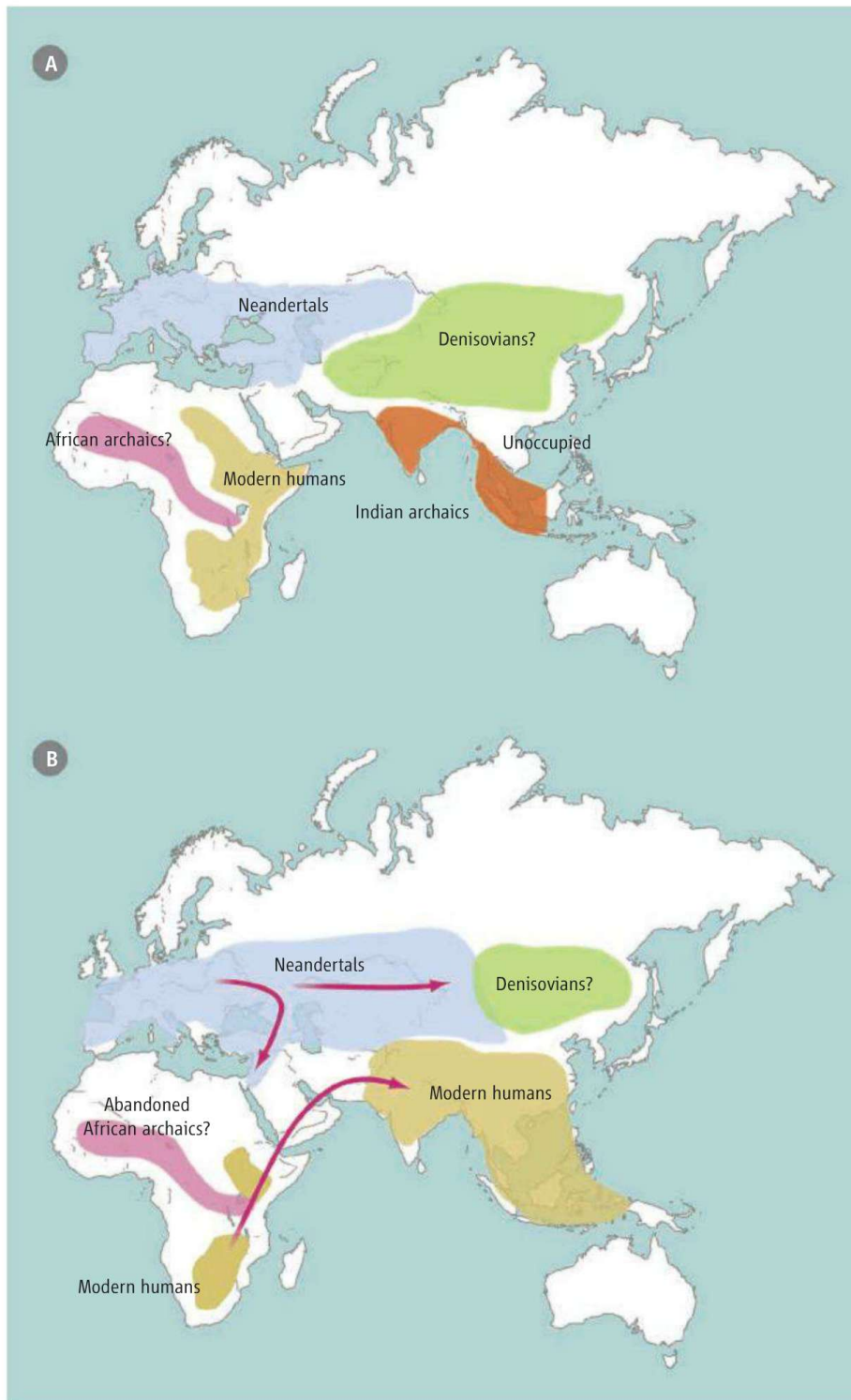
report their high resolution sampling of 1204 Sardinian men, yielding 11,763 phylogenetically informative and male-specific single-nucleotide Y-chromosome polymorphisms (MSY-SNPs), and generate a putative estimate of 180,000 to 200,000 years for the point at which all these and other human paternal lineages coalesce. In a separate study on page 562, Poznik *et al.* (2) detail their methods using sequences from 69 males drawn from nine populations, covering 9.99 million loci on the Y, and conclude that the most recent common paternal ancestor lived 120,000 to 156,000 years ago. These papers further confirm an earlier sequencing study (3) of 36 male donors that pushed the ancestral Y back to 115,000 years

Sampling of the human Y chromosome eliminates the curious disparity in ages of our last common male and female ancestors.

before present (yr B.P.), using almost 6800 variants shared by two or more men. This is roughly the same as the dates derived on the basis of mitochondrial genome analysis for the most recent common maternal ancestor (4). So now it seems that a population giving rise to the strictly maternal and strictly paternal portions of our genomes could have produced individuals who found each other in the same space and time.

While the papers of Francalacci *et al.* and Poznik *et al.* are elegant, careful analyses, the general public is more familiar with mitochondrial and Y-chromosome analyses in the context of population-based comparisons for assigning parentage or assessing continental origin (so-called ancestry

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Ancestors on the move. Using Y-chromosome data from modern human males to date the most recent common paternal ancestor will ultimately help to constrain demographic models of past hominin population locations and migrations. (A) Possible distributions of different populations at ~190,000 B.P. As described in the text, Y-chromosome analyses might help explain the timing of cultural changes seen with regard to microblade tool use and the disappearance of archaic forms of hominins from India, generating the distribution seen at 71,000 B.P. (B). [Adapted from (13)]

generations. For example, Niederstätter *et al.* (6) recently found that efforts to look at bio-geographical origins were complicated by the fact that changes in a 37–base pair region of the Y chromosome occurred more often among thousands of Austrian men than could be accounted for by simple step-wise mutations.

For most biologists, the analysis of SNPs simply provides evidence of population subdivision in the branching patterns of our long-dead ancestors, and this can offer an overwhelming sense of our geographical roots that some will find appealing. However, for social scientists pondering the social consequences of such disclosures surrounding biological diversity in humans, there can be instant recoil at past misguided efforts to use genetics to justify racism. While some have looked at genetic basis of disease susceptibility in the context of migrations of human populations (7), there is always the danger of confusing the effects of selection driven by the environment compared to the genetic history of the populations in question. Indeed, some researchers have concluded that human racial classification is a continuing social construct and not a biological reality at all (8).

On a grander scale of history, discovery bias has been a consistent problem in using SNPs (9), or paleoanthropology, to reconstruct the past. So it is good news that these two new papers provide fresh evidence, using between them a diverse set of data that will allow the consideration of alternative demographic models of hominin migration. The idea that culturally modern humans pulsed out of Africa only 50,000 to 60,000 years ago (10) is widely promulgated, although earlier proposed calibrations of mutation rates for the Y chromosome (11), as well as whole-genome analysis of mitochondrial DNA (12), were consistent with other models of more ancient possible migrations involving anatomically modern humans. Eventual ecological displacement of temperate zone archaic pop-

testing). Maternally (i.e., mitochondrial) and paternally (male-specific regions of the Y) inherited genomes are used in ancestry testing because they present such simple models of genetic inheritance. But they can sometimes oversimplify the search for a genetic homeland and family tree. When using Y-chromosome markers, a grandfather to father to son pattern of inheritance

can be easily reconstructed, both solving and raising issues regarding paternity, as seen in the case of the Jefferson family and Sally Hemings's descendants (5). But SNP markers can undergo a type of genetic recombination called gene conversion that complicates simple Y-chromosome typing and the estimation of mutation rates, leading to spurious conclusions when extrapolated over

ulations, both Neandertal and Denisovan, by modern humans has been put forward to account for discordancy in tool traditions from archaeological research at sites in central India (13), where microblade technology had been in use only since 45,000 years ago, as compared to sites in Africa, China, and Malaysia. In these latter locations, the hominin populations appeared to be culturally modern (in terms of the tools they were making) and well adapted to the emerging localities, suggesting that the time frames implied by a short Y chronology allows insufficient time for inva-

sion and settlement. More broadly, marine isotope 5-calibrated dates in the range of 85,000 to 130,000 yr B.P. suggest that modern humans were on the move between tropical and subtropical zones during the periods when climate oscillated in the temperate regions they would later successfully reinvade, leaving us as their legacy.

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MOLECULAR BIOLOGY

Is There Social RNA?

Peter Sarkies and Eric A. Miska

Our understanding of the forms, functions, and movement of RNA continues to expand. Not only can RNA control gene expression by multiple mechanisms within a cell, it appears to travel outside the cell within an organism as well. This raises the interesting question of whether the RNA world extends beyond the boundaries of the organism. Can RNA traffic integrate an organism into its environment—is there “social RNA”? Examining the mechanism of RNA interference (RNAi) may be a good route for seeking the answer.

In many eukaryotic cells, exposure to double-stranded RNA (dsRNA) can initiate an RNAi response that generates small interfering RNA (siRNA). These are potent silencing molecules that use base-pairing to recognize genes with sequence similarity to the original double-stranded trigger (1, 2). Moreover, many organisms, including mustard cress and roundworms, possess mechanisms to move siRNAs between tissues (3, 4). So far, research into the functions of RNAi has focused on its role within an organism—in antiviral defense or in silencing repetitive DNA sequences in the genome, for example. In the model nematode *Caenorhabditis elegans*, however, molecular mechanisms facilitate trafficking

of functional RNA to and from cells. This extends the RNAi response outside of the cell and possibly even outside of the organism. The functional importance of either for *C. elegans* in the wild is still unknown. However, successfully investigating such roles could be achieved by analyzing nematodes in their natural habitat, for which ecological characterization is more advanced than for the laboratory workhorse *C. elegans*.

One of the most remarkable features of RNAi in *C. elegans* is that feeding these animals dsRNA can silence endogenous genes. This response differs from nonspecific inflammatory responses to dsRNA in mammalian cells because only genes with matching sequence to the ingested dsRNA will be silenced (5, 6). A specific pathway

that takes up long dsRNA (~200 to 500 base pairs) from the gut lumen involves the channel protein SID-2 (7, 8). Inside the cell, dsRNA is cleaved by the endoribonuclease Dicer (DCR-1) to generate ~23-nucleotide RNAs, known as siRNAs. siRNAs are

bound by Argonaute proteins, and the resulting complex targets messenger RNA for degradation. In addition, RNA-dependent RNA polymerase enzymes amplify the trigger, thereby bolstering its silencing effect on target genes (9). Another dsRNA-selective channel, SID-1, subsequently allows the silencing RNA signal to spread throughout the animal (10). The signal can even reach the germ line, thus instigating a transgenerational response (11–13).

The idea that RNA can be transferred between organisms and function in communication and environmental sensing is discussed.

Exploiting the SID pathway enables the function of almost any gene to be examined simply by making a bacterial strain expressing dsRNA that matches the gene of interest—a great tool. But the broad implications of the response that this pathway elicits have not resonated widely, in part because its function in the normal *C. elegans* life cycle is mysterious. What possible use could there be for a pathway that takes up RNA from the environment and uses it to silence endogenous genes?

An attractively simple idea is that *C. elegans* might respond to dsRNA that is naturally produced by the bacteria it consumes. This would allow *C. elegans* to mount an RNAi response against bacterial RNAs that enter the gut. siRNAs produced by cleavage of a bacterial dsRNA trigger could target endogenous genes, redirecting gene expression programs in response to different diets. Although a plausible model, there is no clear evidence yet that this occurs. A noncoding RNA produced by certain *Escherichia coli* strains might cause gene expression changes via RNAi in *C. elegans* (14). However, mutations in SID pathway genes do not obviously compromise fitness under laboratory conditions, which suggests that “environmental RNAi” is not important for growth on *E. coli* in general. In the wild, *C. elegans* probably feeds on bacteria growing on rotting fruit (15) and therefore encounters multiple species of microbes, so deeper sampling of ecologically relevant bacteria might provide insight into the role of SID-encoding genes. Our understanding of the

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Podcast interview with author Eric Miska (http://scim.ag/ed_6145).

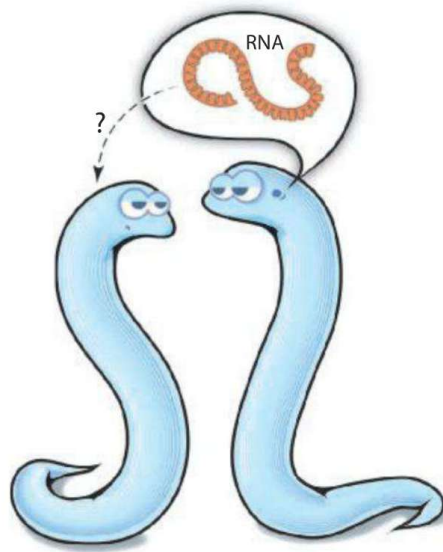
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natural environment of *C. elegans* is limited, making this difficult.

An analysis of related nematodes with ecology that is better understood may provide some hints. Many parasitic nematodes undergo well-characterized life cycles involving two different hosts (16) (e.g., *Brugia malayi*, the causative agent of lymphatic filariasis, is transmitted between humans by mosquitoes) or both free-living and parasitic stages (e.g., the hookworm *Nippostrongylus brasiliensis* goes through two larval molts, feeding on rat fecal matter before reinfesting a rat host). RNAi has been demonstrated experimentally in parasitic nematodes of plants and animals (including human) (17). It is conceivable that parasitic nematodes could use host RNA as a trigger to regulate endogenous genes in response to life cycle cues.

If a parasitic strategy exists, perhaps hosts exploit it by producing dsRNA to attack parasites. Indeed, resistance to root-knot nematodes can be engineered by making transgenic plants that express dsRNA against an essential nematode gene (18). This points to the exciting possibility of an “RNA arms race” between host and parasite. In support of this, the genes involved in taking up RNA from the environment in *C. elegans* appear to be evolving rapidly. For example, the closely related nematode *C. briggsae* has lost the gene *sid-2* and is insensitive to feeding with dsRNA-expressing bacteria (7). However, expressing *C. elegans sid-2* allows *C. briggsae* to take up dsRNA from its environment. Perhaps the ancestor of *C. briggsae* was exposed to bacteria that exploited the *C. elegans* uptake pathway, resulting in selective pressure to lose the RNA uptake mechanism. But *sid-2* homologs may not be the only way by which RNA is taken up from the environment. One example is the nematode *Meloidogyne incognita*, which, despite responding to dsRNA administered through feeding, has no *sid-2* homolog. Thus, environmental RNAi is likely to occur in nematodes without obvious *sid-2* homologs (19).

In addition to the uptake of long dsRNA, siRNAs (downstream of DCR-1 activity) can move within *C. elegans* and can be amplified to generate abundant secondary siRNAs in the recipient tissues (20). This means that if the animal were exposed directly to small RNAs from its environment, these might generate an RNAi response in the absence of a dsRNA trigger. Such a process could have interesting consequences. For example, *C. elegans* could spread an antiviral RNAi response within a population to generate herd immunity. Exactly how small RNA



mobility occurs within *C. elegans*—the precise species that moves, as well as its mechanism of secretion and uptake—is unknown. When elucidated, the process should inform whether small RNAs can survive outside of the parent organism.

Small RNAs, in particular microRNAs (miRNAs), can be secreted from mammalian cells (21). Different body fluids, including blood, tears, and saliva, contain miRNAs, either free or contained within vesicles (exosomes) (22). Because miRNAs are functionally very similar to siRNAs, and indeed may have evolved from the siRNA pathway, it is plausible that comparable mechanisms of small RNA secretion might be used for siRNAs. Determining whether nematodes incorporate small RNAs into exosomes would help to clarify the range of RNA mobility, as well as the evolution of the mammalian miRNA secretion pathway.

A highly debated issue that is not yet resolved convincingly is whether secreted miRNAs from mammalian cells have an effect in recipient cells. One crucial point is that mammals do not possess amplification pathways to allow small amounts of an RNA taken up from the environment to trigger a larger response within the organism. Therefore, the miRNA itself would have to be taken up in sufficient amounts to have an effect, either with an Argonaute protein from the donor or through incorporation into the recipient cell's Argonaute complexes. Indeed, secreted miRNAs in conditioned medium from cells overexpressing miRNAs can down-regulate target genes in recipient cells. This secretion depends on GW182 proteins, which interact with Argonaute pro-

teins (23). GW182 proteins could thus move with miRNAs to facilitate their effect.

There is a high degree of sequence conservation between miRNAs of different organisms. This could be important in host-parasite interactions, for example, such that miRNAs secreted from a parasite could manipulate host gene expression programs. Such cross-species traffic of miRNA has been observed. miRNAs from edible plants are found in human bodily fluids, which suggests that miRNAs might be assimilated into mammalian miRNA pathways (24). However, such studies suffer greatly from potential contamination artifacts and have thus proved controversial (25). Lab model systems such as *C. elegans* are crucial for rigorously establishing the transfer of small RNAs between organisms and for addressing mechanistic details of small RNA secretion.

Is there an extensive life for RNA molecules outside of the cell, and even outside of the organism that produced them? Investigating possible examples of “social RNA” presents an exciting avenue for future research, as the implications of such a phenomenon would be far-reaching indeed.

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EVOLUTION

Why Male Mammals Are Monogamous

Peter M. Kappeler

Monogamy has long fascinated scientists and the general public alike (1). Its occurrence in fellow mammalian species has puzzled evolutionary biologists (2–4). Male mammals have a much higher potential for producing offspring per unit time than females, making it necessary to identify selective advantages that would more than compensate for the loss of potential reproduction suffered by males that confine their reproductive activities to a single female. On page 526 of this issue, Lukas and Clutton-Brock (5) show that the costs of monogamy for males are not compensated by fitness benefits through paternal care. Instead, by forming pairs, males overcome disadvantages that arise because ecological factors promote wide spacing of individual females.

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In birds and some species of teleost fish, anurans, and arthropods, direct paternal care for offspring is possible and enhances offspring survival, and selection, therefore, favors male reproductive strategies that promote monogamy. In mammals, however, internal gestation and subsequent lactation are essential aspects of parental care limited to females. These physiological constraints reduce the rate at which females can produce offspring compared with males (6). Thus, because male mammals that bond with a single female forgo additional mating opportunities, monogamy as a male reproductive strategy requires explanation.

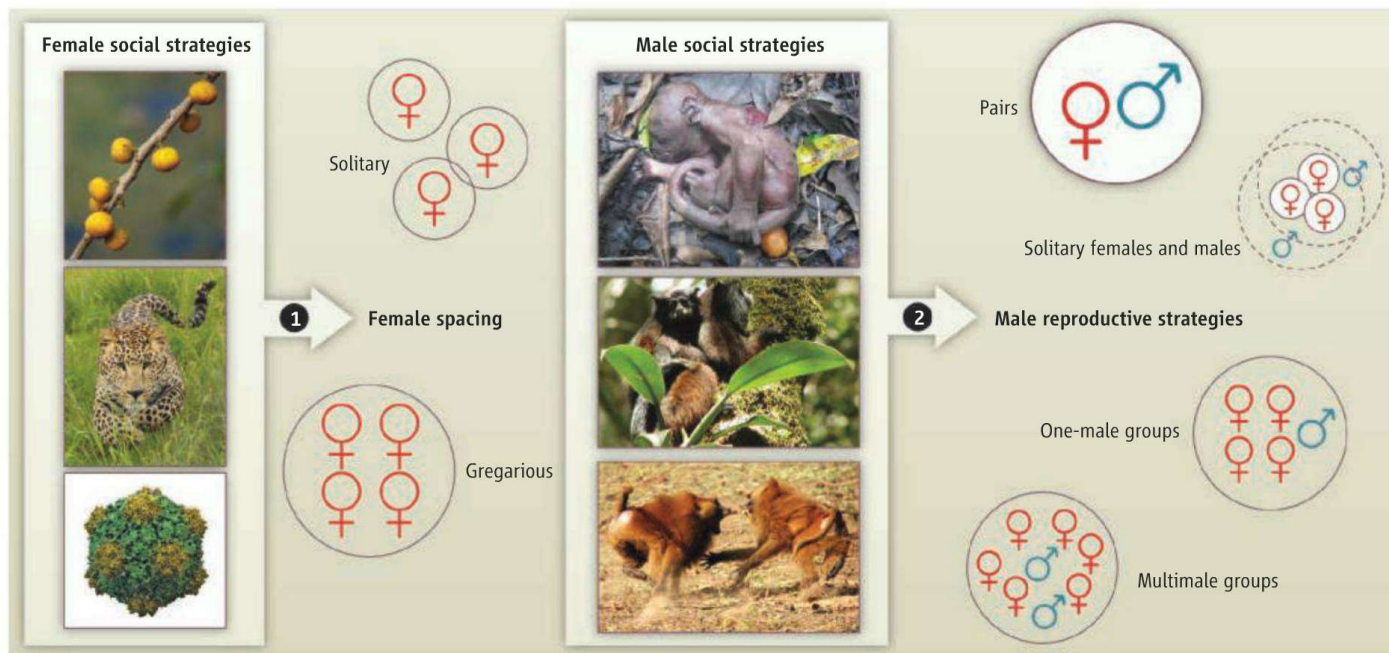
Of course, females pursue reproductive strategies of their own, which are not limited by the number of mates but by access to resources and the availability of paternal care. Thus, characteristics of their diet and other ecological factors determine the distribution of females in space (see the figure). Depending on the balance between the costs and benefits of gregariousness, females either

Social monogamy evolved many times across mammals as a male mating strategy in species where females are widely spaced for ecological reasons.

form groups or lead solitary lives in individual home ranges that may overlap with those of neighboring females.

Thus, female mammals set the ground rules, and males map themselves onto their distribution (7). Because the number of mates limits male reproductive success, males are sensitive to the spatial distribution of females (8), as well as to the degree of synchrony among their receptive periods. When faced with solitary females, males have two options: They can either roam widely, trying to cover and defend the ranges of several females, or they can associate and settle with one female and form a pair.

The big question from the male perspective is whether pair-living is imposed upon males by the spatial distribution of females or whether selection favors it despite the costs of forgone mating opportunities. Many hypotheses proposed to explain the evolution of monogamy in mammals have been refuted (3, 9); others turned out to be more appropriate for birds or other taxa (7, 10). At present,



Evolution of mammalian sociality. The distribution and abundance of food resources, predators, and pathogens (left) determine whether females of a given mammalian species are solitary or gregarious. Male social strategies are determined in a second step by infanticide risk, options for parental care, intrasexual

selection, and the spatiotemporal distribution of receptive females. Lukas and Clutton-Brock show that of these factors, it is the female spatiotemporal distribution that determines whether mammals form pairs. When solitary females are clumped or when females are gregarious, other social organizations evolve.

there is contradictory evidence concerning the female spacing (4, 11) and paternal care hypotheses (9, 11, 12). Moreover, most previous studies assumed that pair-living species had solitary ancestors, but a recent study of primate social evolution indicated group-living ancestors (13).

To resolve these questions, Lukas and Clutton-Brock have compiled socioecological and life-history data for more than 2500 species of mammals from all orders. Using phylogenetic reconstructions, they identify 61 independent transitions to social monogamy, all but one from ancestral species with solitary females. Although paternal care characterizes most socially monogamous species today, their analyses strongly suggest that it evolved after pairs had formed, excluding paternal care as a dominant selective force favoring the evolution of mammalian monogamy. Their analyses also exclude a reduced risk of male infanticide as a main driver of the evolution of pairs, rejecting the hypothesis that paternal investment takes the form of a protective association with mother and infant.

The authors also show that pair-living species living today occur at lower densities and with less range overlap with neighbors

than females in solitary species. It is therefore likely that pair-living females are more widely spaced out for ecological reasons and that they experience intense female feeding competition. Thus, during the origins of mammalian pair-living, males were presumably coerced into this type of social organization because they were limited in their ability to monopolize multiple, widely spaced females; the benefits of paternal care and infanticide protection only subsequently stabilized its maintenance.

By relying on an astounding sample size and clear definitions, Lukas and Clutton-Brock are able to explain many contradictory results of previous studies. Their study also provides resounding support for the socioecological model, a theoretical framework for evolutionary social systems research that has received some criticism (14). However, the study also raises new questions. For example, why is monogamy so unevenly distributed among mammalian orders? Can its absence in most orders be explained by (mostly unknown) dietary adaptations alone? Why are pair-partners in some species closely associated year-round, whereas in others, they only meet for mating once a year? And how common are

extra-pair copulations or intraspecific variation in social organization in different species, and what are the sociogenetic consequences? Some of these questions can also be studied in humans (1), where monogamy did not evolve from solitary ancestors and where additional factors promoting pair-bonding and monogamy must, therefore, be sought (15).

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CHEMISTRY

Isothermal Water Splitting

Martin Roeb and Christian Sattler

The potential of solar radiation as an infinite resource of renewable energy is widely recognized. The challenge is to convert it as efficiently as possible into useful energy forms such as hydrogen or liquid carbonaceous fuels. Thermochemical cycles are a number of consecutive chemical reactions (≥ 2) that lower the maximum temperature compared to a single chemical reaction. Typical temperatures for these cycles to reach full conversion range from 800°C to 2000°C. The necessary solar heat can be generated by concentrating optics that direct the solar radiation on a single point—in solar towers or central receiver systems. To use the solar power efficiently and economically, losses must be minimized, with major factors being the minimization of re-radiation and the reduction of gases that are used to transport the reactant. As pointed out by Muhich *et al.*

(1) on page 540 of this issue, a particularly important factor in this respect is the minimization of temperature differences between reaction steps. However, the necessary temperatures are high, and it has yet to be proven that the materials and components are stable over a long time to make the processes economically attractive.

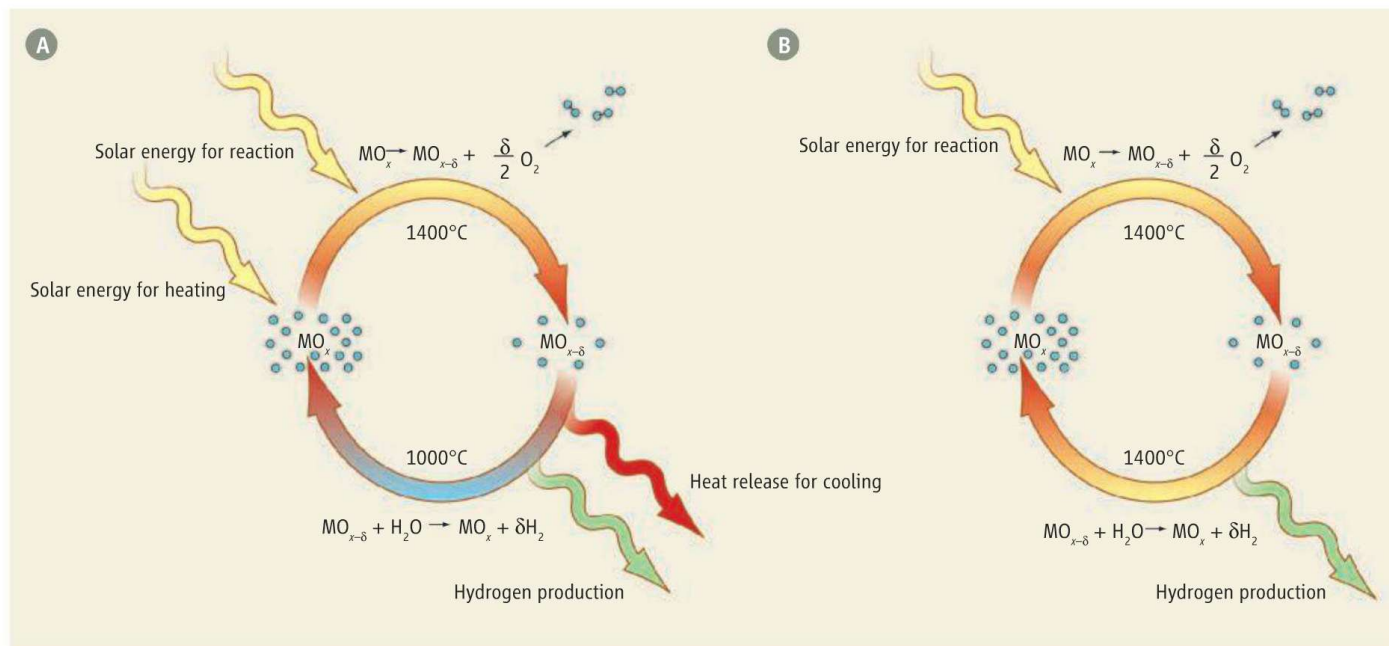
For the success of such processes, it is considered crucial to find suitable redox pairs, multivalent metal oxides capable of reversibly incorporating oxygen atoms. Some materials (e.g., Cu₂O/CuO or CoO/Co₃O₄) do that perfectly if the oxidation is done by air, but it is much more challenging if the oxidation is done by water or CO₂, because those are much more stable molecules than oxygen. The reactions involved are on the edge of being feasible and practicable because high temperatures are needed and the gas-solid reactions cannot be carried out continuously (the reactive solid is consumed as the reaction proceeds). Therefore, other means to drive the reactions have to be found.

Process operation at constant temperature may enhance the solar-thermal production of hydrogen from water.

It is known that a reaction (thermal reduction) and the reverse reaction (water/CO₂ oxidation) are favored by different conditions. Thermal reduction requires high temperatures and low oxygen partial pressures, whereas oxidation favors low temperatures and high partial pressures of steam. However, the reaction kinetics needs to be considered, which imposes restrictions on the temperature of the water oxidation: too low and reaction becomes too slow to be applicable.

Most efforts have so far concentrated on the temperature as the main parameter to ensure the necessary driving force for the two reactions involved. Thermal reduction is carried out at as high a temperature as needed to reach appropriate chemical conversions. For most of the metal oxides, this means close to 2000°C. To get that temperature down, reaction conditions with low oxygen partial pressure are applied, shifting the thermodynamic equilibrium toward the products and thus enabling high conver-

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Energy conversion. Scheme of (A) temperature swing operation; and (B) isothermal operation. ($\text{MO}_x/\text{MO}_{x-\delta}$: oxidized/reduced form of metal oxide).

sions at lower temperatures, ensuring less thermal losses. But still, the temperature needed to effectively reduce the metal oxides of interest (e.g., CeO_2 and NiFe_2O_4) is 1400°C and above (2).

For water oxidation, the temperature must be optimized. Thermodynamics favor low reaction temperatures, but the kinetics need to be fast enough and so forbid temperatures that are too low. Therefore, the entire process (thermal reduction and water oxidation) is carried out by a temperature swing operation with a temperature difference of about 400 K (see the figure, panel A). In parallel to this, the gaseous feed to the metal oxide has to swing between steam and inert purge gas.

A number of process and reactor concepts achieve such a temperature swing. Some are based on transporting metal oxide particles in the form of fluidized beds or moving beds (3–5), where the particles are conveyed from hotter sections of the reactors where thermal reduction takes place to colder ones to allow for the water oxidation, and returned after completion. Others suggest doing this by using monolithic porous structures made of the active metal oxides and turning or moving them such that they alternately move through hot and cold sections (6, 7). A third group of approaches suggests fixing the metal oxide and alternating the solar heat flux by focusing/defocusing of the mirrors (8, 9).

The importance of keeping and recovering the heat content of the metal oxides has been stressed (10). If the heat recovery is not done sufficiently, the associated losses can be

detrimental for the efficiency and economics of the process. Muhich *et al.* suggest carrying out both reaction steps at the same temperature. Heat losses due to the temperature swing are thus avoided (see the figure, panel B). Heat recovery is simplified by isothermal operation but becomes even more important because the process concept is based on increasing the temperature for the exothermal water oxidation. Heat rejection at a temperature of 1400°C needs to be avoided as far as possible because the efficiency would suffer severely. What also needs to be addressed in the future to further validate the concept of isothermal thermochemical water splitting is the potential competition of water oxidation and thermal reduction at such a temperature in the sense of avoiding simultaneous production of hydrogen and oxygen.

To increase the driving force of the reaction, most approaches apply the principle of least constraint. In flow-through systems, a stream of gaseous reactants passes through a bed, a porous structure, or across the surface of a solid body of metal oxide. A surplus of reactants is thereby supplied and products removed continuously from the reaction zone, thus shifting the chemical equilibrium and driving the reactions to completion over the long term. The main challenge with this kind of operation is to maintain sufficiently high reaction rates because the overall efficiency of solar plants crucially depends on the capability of absorbing as much radiation as possible and to convert as much of it as possible into some useful form. In the present case, that means a fast and constant conversion of highly

concentrated solar radiation into hydrogen or syngas (synthetic gas). Therefore (and this is in particular true for flow-through systems), metal oxides are needed that exhibit fast-reaction kinetics for both thermal reduction and water oxidation.

The cost of solar processes and thus the price of their product (hydrogen or syngas) are driven by the cost of the solar collectors. The main development target for the present application is to identify the process with the least collector area needed to generate one kilogram of hydrogen, and thus maximize the solar-to-fuel efficiency on an annual basis. The efficiency would need to be 20% or above to be superior to solar-powered low-temperature electrolysis (11). Isothermal water splitting appears to be a viable approach showing promise to reach this goal.

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INTRODUCTION

Once and Future Climate Change

ANTHROPOGENIC CLIMATE CHANGE IS NOW A PART OF OUR REALITY. EVEN THE MOST optimistic estimates of the effects of contemporary fossil fuel use suggest that mean global temperature will rise by a minimum of 2°C before the end of this century and that CO₂ emissions will affect climate for tens of thousands of years. A key goal of current research is to predict how these changes will affect global ecosystems and the human population that depends on them. This special section of *Science* focuses on the current state of knowledge about the effects of climate change on natural systems, with particular emphasis on how knowledge of the past is helping us to understand potential biological impacts and improve predictive power.

Four News stories focus on past and future impacts of climate change and the techniques that researchers are using to study them. Gibbons examines the role of climate variability in hominin evolution in Africa, and Pennisi profiles an effort to use sediment cores to document that variability. Kintisch explores whether coastal wetlands will be able to outclimb rising seas. And Pennisi offers a snapshot on the use of historical photographs to study climate impacts.

Four Reviews discuss recent research on the current and future effects of climate change as informed by our understanding of changing climates in the paleorecord. Diffenbaugh and Field review the physical conditions that are likely to shape the impacts of climate change on terrestrial ecosystems, showing that they will face rates of change unprecedented in the past 65 million years. Norris and colleagues review the Cenozoic history of oceanic change; despite some short-lived past analogs, the oceans will also experience more rapid change than ever before. Turning to ecology, Blois and colleagues discuss how climate changes can affect biotic interactions and how these insights might inform our understanding of future interactions. Moritz and Agudo discuss the prospects for species survival, weighing the evidence for persistence versus catastrophic decline.

Three Reviews focus on more specific impacts of climate change. Its influence on infectious disease is considered by Altizer and colleagues, who use examples from a wide range of host-pathogen systems to assess whether we are close to a predictive understanding of climate-disease interactions and their potential future shifts. Wheeler and von Braun assess the prospects for human food security, with particular attention to potential impacts on food supply in the world's more impoverished countries. Finally, Post and colleagues take a regional focus, reviewing the ecological consequences of current sea ice decline in the polar regions, the part of the world where the reality of changing climate is perhaps at its most stark.

— CAROLINE ASH, ELIZABETH CULOTTA, JULIA FAHRENKAMP-UPPENBRINK,
DAVID MALAKOFF, JESSE SMITH, ANDREW SUGDEN, SACHA VIGNIERI

Natural Systems in Changing Climates

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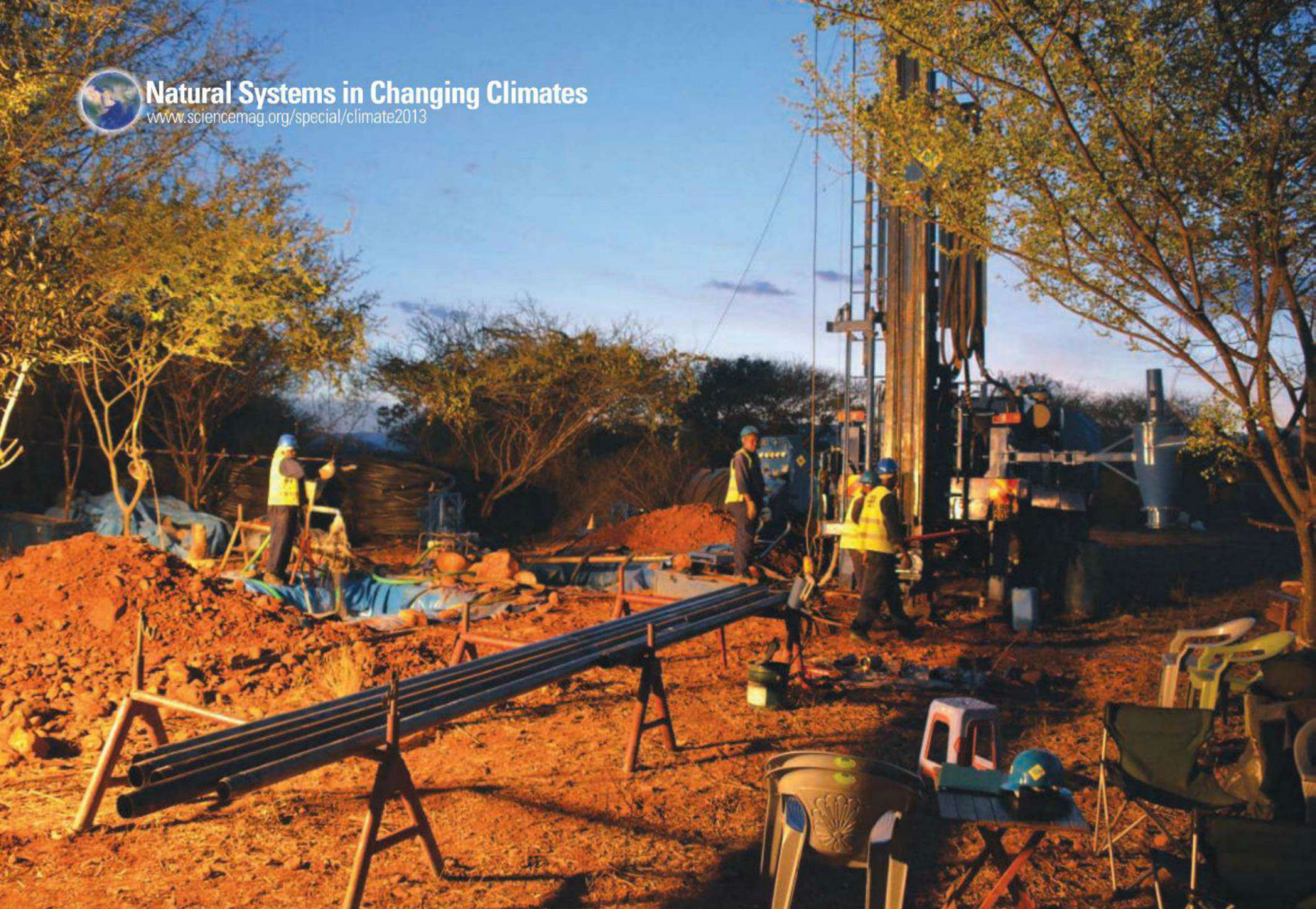
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Wet to dry. Kenya's Lake Magadi, now alkaline and mostly dry, once teemed with freshwater fish; a core drilled here will reveal ancient climate swings.

Science



HUMAN EVOLUTION

How a Fickle Climate Made Us Human

Researchers are drilling for clues to how dramatic changes in African rainfall and vegetation shaped our species

Humans, like children, are the products of their environment. The famous anatomist Raymond Dart recognized that back in 1925, when he described the first hominin skull found in Africa. The evolution of this “Man-Ape,” he wrote, markedly differed from that of earlier apes. While apes lolled about in “luxuriant” tropical forests that posed relatively few survival challenges, the “Man-Ape” had to compete for scarce food and water with saber-tooth tigers and other dangerous beasts of the arid savanna—and ended up sapient. “For the production of man a different apprenticeship was needed to sharpen the wits and quicken the higher manifestations of intellect—a more open veldt country,” Dart wrote.

This “savanna hypothesis” suggested that as a drier climate caused grasslands to spread, our ancestors moved out of the trees and began walking upright in order to spot predators and prey in the waist-high stems. That freed their hands to use tools and spurred the development of big brains.

Today, no serious paleoanthropologist believes that particular evolutionary tale. But Dart’s hypothesis was the first of many to propose that shifts in climate and environment made humans who we

are. The idea has become practically received wisdom even though there has been little direct evidence to support or falsify it. True, researchers have extracted precise records of past climate from sea-floor sediments and ice cores. And they have noticed that fossils and environmental clues on land also suggest that some climate shifts coincide with changes in human ancestors.

But correlation is not causation, and only “a circumstantial case” has been made for climate as the engine driving human evolution, says paleoceanographer Peter deMenocal of Columbia University’s Lamont-Doherty Earth Observatory in Palisades, New York. Even correlation can be elusive: Syncing fossil discoveries with offshore climate records thousands of kilometers away has proven challenging. Most stories of how environmental change shaped our evolution have been “mainly fantasies of the past,” says paleoanthropologist Andrew Hill of Yale University. “They are not proven.”

That is beginning to change, however, as researchers deploy new tools to reconstruct climate and environment right where ancient hominins—the ancestors of humans but not other apes—once lived. This summer, for instance, a truck with a drilling rig has worked its way up the Rift Valley of Kenya, extracting sediment cores from dried-out lakebeds next to key fossil sites. Specialists are already analyzing a core drilled last year at Olorgesailie in Kenya (see story, p. 476).

CREDIT: ANDREW HILL/YALE UNIVERSITY

Wellsprings of data. Researchers are drilling deep into Africa's Great Rift Valley to get detailed climate data.

Such detailed, localized work may put some theories to rest and breathe new life into others, including a revived version of the savanna hypothesis.

"For the first time, we'll be able to frame the question as: 'How did hominins respond to the environment they lived in' rather than as responses to global or Northern Hemisphere events," says geologist Craig Feibel of Rutgers University in New Brunswick, New Jersey.

Grass roots

If the past is a foreign country, as historians say, then the prehistoric landscape is an alien world. Using a host of climate indicators, geologists have uncovered evidence of dramatic events that changed the planet in the past 7 million years, showing that the African landscape has evolved as dramatically as the anatomy of hominins. In the words of Dart, our ancestors' "eyes saw, their ears heard, and their hands handled," terrain far different from today's.

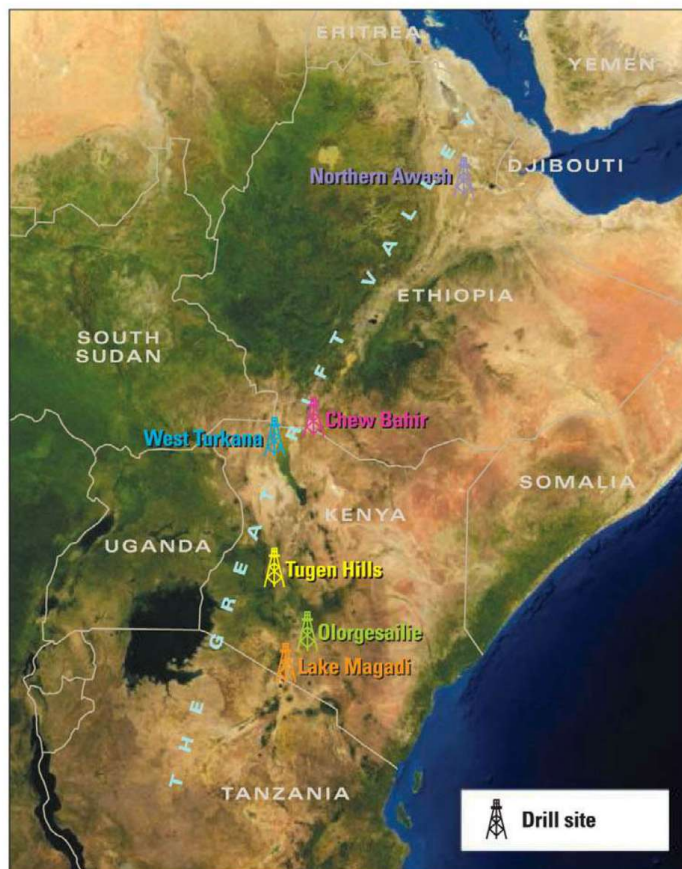
About 4 million years ago, for example, the Turkana Basin in Kenya—home to seven hominin species, including our direct ancestor *Homo erectus*—was covered by a vast inland sea, three times bigger than the lake there today. At the continent's northern edge, the Mediterranean Sea dried up between 5 million and 6 million years ago, decreasing the circulation of moisture over Africa and Europe, according to a 2010 National Research Council report. This may have amplified a cooling and drying trend that had already started in Africa 6 million to 8 million years ago, according to oxygen isotopes from marine and ice cores.

That long-term drying trend, which began at about the time when the human and chimp lineages diverged, tempted some researchers in the 1990s to propose a revised savanna hypothesis: Drier, cooler climates thinned the forests of Africa, perhaps driving hominins out of the woods to scurry upright across open grasslands between patches of trees in search of food.

Then, new fossil discoveries challenged that idea. Paleoanthropologists found three very early, upright hominins that, according to clues left in the fossil sites, apparently lived in the woods between 7 million and 4 million years ago: *Ardipithecus ramidus* from Aramis, Ethiopia; *Orrorin tugenensis* from the Tugen Hills of Kenya; and *Sahelanthropus tchadensis* from Chad. For example, researchers found fossils of *Ar. ramidus* with fossilized wood and seeds, and near fossils of woodland monkeys, parrots, and snails. With hominins walking in the woods, the savanna hypothesis bit the dust.

Now, however, a half-dozen geologists are resurrecting the importance of grasslands in human evolution once again. This time, however, the theory has to do with how human ancestors used the savannas for food, not hunting and rambling.

The renewed savanna hypothesis has its roots in evidence that as African climate dried out 6 million to 8 million years ago, the makeup of plant communities was also shifting, says geochemist Thure Cerling of the University of Utah in Salt Lake City. That's because the amount of carbon dioxide in the atmosphere began to decline 10 million years ago, giving a boost to plants that use the more efficient C_4 photosynthetic pathway; most of those plants are grasses and sedges, whereas woodland plants such as trees and shrubs usually rely on the older C_3 pathway. Grasses and other C_4 plants went from carrying out 1% of the photosynthesis in the tropics 10 million years ago to 50% today.



As went plants, so went the animals that grazed on them: By 6 million years ago, C_4 grasses had replaced C_3 plants as the most significant component in the diet of African grazers, Cerling says, according to studies of carbon isotopes in the tooth enamel of horses, elephants, antelopes, and other animals.

This suggests that hominins were born when grasses were on the rise. In fact, Cerling and his colleagues think that the first hominins had more grass in their environment than initially proposed—40% to 60% of the vegetation at nine *Ar. ramidus* fossil sites was C_4 plants, Cerling suggests (*Science*, 28 May 2010, p. 1105).

Recent data now show that later hominins responded to the rise of grasses by broadening their diets. Species that arose more than 4 million years ago, including *Ar. ramidus* and the oldest australopithecine, *Australopithecus anamensis*, subsisted on an apelike diet of at least 90% leaves and fruits from C_3 plants, Cerling and his colleagues reported in June in the *Proceedings of the National Academy of Sciences*. By 3.5 million years ago, a descendant of *Au. anamensis*—*Au. afarensis*, whose most famous member is the skeleton named Lucy—apparently adapted to the widespread grasslands by also munching on many C_4 plants, according to Cerling's analysis of carbon isotopes in the tooth enamel of seven hominin species. *Au. afarensis*—a leading candidate for the ancestor of *Homo*—and another hominin, *Kenyanthropus platyops*, still ate mostly C_3 woodland plants, but about 22% of their diet was also made up of these C_4 plants, making them the hominins with the most varied menu. Their meals included grasses and sedges such as water chestnuts and papyrus and perhaps animals that fed on those plants.

This appetite for grasses apparently left its mark on *Australo-*



Where the hominins roamed?

New data suggest that grassland environments were important in hominin evolution.

pithecus anatomy: Most members of the genus had much larger molars and premolars than earlier hominins. Such big choppers would last longer than small ones when chewing on gritty grasses and abrasive sedges.

Given that grasses were abundant in all hominin habitats for so long, “the savanna hypothesis is alive and well,” Cerling says. The grasslands may have had other evolutionary impacts, too, although the revived savanna hypothesis is so new that paleoanthropologists are just beginning to consider it. They note that the earliest hominins walked upright, but with a variety of odd gaits; the savanna may have favored adaptations for more effective walking and running, such as an arched foot and nonopposable big toe, that led to the typical human gait (*Science*, 11 February 2011, p. 750). “Cerling’s data are causing us to refocus on the link between environment and adaptation,” says paleoanthropologist Carol Ward of the University of Missouri, Columbia. “But we still have more questions than answers.”

Cerling’s savanna hypothesis “is not Dart’s savanna hypothesis,” says Richard Potts, a paleoanthropologist at the Smithsonian Institution in Washington, D.C. That’s partly because geologist Cerling uses a broader definition of “savanna” than many paleoanthropologists. But it’s also because the hominin most

Out of the Kenyan Mud, an Ancient Climate Record

MINNEAPOLIS, MINNESOTA—When Richard Potts, Anna Behrensmeyer, Alan Deino, and Richard Bernhart Owen get together, it’s usually at camp at Olorgesailie in Kenya’s Great Rift Valley. The paleoscientists labor for hours in the hot sun, chipping away at exposed rock outcrops to develop a timeline for artifacts and other relics of the human ancestors who once lived nearby. But on a cold spring day earlier this year, they gathered with a dozen other researchers in a small lab at the University of Minnesota to analyze something quite different: 190 meters of mud, sand, and gravel cored last year, from what they hoped was once a lakebed 20 kilometers from their outcrops.

The team had never tried to drill a core before and hadn’t even known for sure that a lake ever covered the area. But as the researchers kicked off their “core sampling party,” they hoped that the mud would resolve into thousands of distinctive layers, each representing a different climate regime, ultimately reaching back 500,000 years. The goal: to test ideas about the role of climate variability in human evolution, by getting a continuous record of climate indicators from a place where hominins lived and died (see story p. 474). The project “has high potential to reconstruct the long-term history of environmental change,” says

team member Vanessa Gelorini, a paleoecologist at Ghent University in Belgium.

Although everyone eagerly anticipated what might be hidden in the 139 half-meter- to 3-meter-long cylinders of sediment, the group was anxious. The work required difficult on-the-spot decisions and taking hundreds of samples—chores best accomplished by everyone working together at the same place at the same time, says Potts, the Smithsonian Institution National Museum of Natural History (NMNH) paleoanthropologist who has led the Olorgesailie project since 1986 and raised \$450,000 in private funding for the drilling and party. But cores don’t come with labels, and everyone had questions. Do we have a truly continuous record? How far back does it go? Can we date the layers? In other words, will this investment give us the data we seek?

Digging for climate proxies

At the field site, Olorgesailie, researchers have been digging out artifacts and fossils since Louis and Mary Leakey first explored it in the 1940s. Since 1986, geologists have tried to extract clues to ancient climates from the outcrops (*Science* 23 March 1990, p. 1407). But the outcrops are not a continuous record of the past: They repre-

sent the period from 1.2 million to 500,000 years ago and then pick up again from 320,000 years ago to the present. Rock formed during the gap has eroded away.

Yet during those missing millennia, humans moved from a culture limited to stone axes to the so-called Middle Stone Age, marked by new tool innovations and perhaps more sophisticated social interactions. Potts proposed more than a decade ago that an increase in the variability of the climate during that time shaped human evolution by ratcheting up hominins’ genetic and phenotypic plasticity, so that they could survive in a broad range of conditions. Those changes prepared our ancestors to eventually spread worldwide.

But Potts’s theory couldn’t be tested without a climate record from the missing years. Where might it be preserved? One possibility was the Koora Valley 20 kilometers to the south, which was connected by a shallow depression to the Olorgesailie site. Potts and his NMNH colleague Behrensmeyer speculated that sediments eroded away at Olorgesailie had washed into the channel and down to the basin. If there had been a lake there, the researchers would be in luck. Lake bottoms typically accumulate sediments year after year, and, hidden from the sun and weather, those

specialized for the grasslands was not on the line leading to *Homo*. The “Nutcracker Man,” *Paranthropus boisei*, used its giant molars to crunch on a diet of 75% C₄ grasses and sedges, according to recent isotopic studies—and it died out about 1.2 million years ago, Lamont-Doherty’s deMenocal notes. They “weren’t the successful ones.”

Burst of speciation

Over the years, other hypotheses connecting human evolution to climate have also come and gone. For example, in the 1980s, Yale’s Elisabeth Vrba suggested that dramatic shifts toward a cooler, drier climate in the East African Rift Valley between 2.7 million and 2.5 million years ago sparked bursts of rapid extinction and speciation in grazers like antelopes, as well as in hominins. But this turnover-pulse hypothesis—so named because pulses of climate change were thought to spark big turnovers in species—faded after later studies showed that the shift in species happened more gradually. Still, the idea’s not dead yet: Arizona State University, Tempe, paleo-anthropologist Kaye Reed has spotted another burst of speciation about 3 million years ago at Hadar, Ethiopia. There, 10 new species, including camels and hoofed grazers, appear just before *H. habilis* replaced *Au. afarensis*.

Although Vrba thought that drying had sparked rapid speciation, other researchers have noticed that new species of hominins seem to appear during wet, humid periods. For example, studies of lake sediments in 10 East African rift basins suggested that the overall cooling and drying trend of the past 8 million years was interrupted by at least three humid periods when deep lakes filled, which tie in with the birth

of new hominins. So did cool, dry climates or wet, humid ones shape the evolution of hominins?

Potts has a different answer: It was all these changes, fluctuating wildly, that produced humans.

Creatures of change

Potts proposed 16 years ago that the key adaptation of the human lineage, manifested in everything from big brains to culture, is adaptability: Individuals who could survive in wet woods as well as dry grasslands fared better than those specialized for one or the other. He argues that the dramatic fluctuations of the ancient African climate shaped human nature, allowing our species to eventually thrive in all sorts of environments worldwide.

Deep-sea cores suggest that “the first appearance of every major genus in our evolutionary history, the origin of every major stone technology, happens to fall in long periods of high climate variability,” Potts says. He ticks off in rapid fire the innovations of such periods: the birth of *Australopithecus*, *H. habilis*, *H. erectus*, *Paranthropus*, and *H. sapiens*, plus the invention of the first stone tools 2.6 million years ago, the creation of more advanced Acheulean tool kits 1.8 million years ago, and the first Middle Stone Age technologies 300,000 years ago. Each event is correlated with a period of high climate variability, such as wet and dry cycles, Potts says. For example, the span between 2.5 million and 2.7 million years, which Vrba noted had such turnover in species, cycled between extremely wet and extremely dry times.

But the evidence for those fluctuations comes from predictions

sediments are often well-preserved. “We didn’t know for sure that there was going to be a lake,” Behrensmeyer recalls. “If it were just a pile of pumice or volcanic ash, it wouldn’t have preserved the environmental signals.”

To test the idea, Potts secured funding from several private foundations. A local drilling company spent September 2012 digging out two cores on the Koora Valley and shipped them here to the National Lacustrine Core Facility (LacCore), a lake core processing and storage lab supported by the U.S. National Science Foundation.

Guest of honor

At the core party, technicians begin slicing each 4-centimeter-wide core section lengthwise down the middle, enabling researchers to mine the sediment for a plethora of indicators of ancient climate. For example, different types of plants fractionate the two isotopes of carbon, C-12 and C-13, differently. So analyses of plant waxes extracted from the sediments can indicate whether dry-adapted grasses or moisture-loving vegetation thrived. Bits of calcium

carbonate, found in some soils, contain isotopes of oxygen (O-16 and O-18) that can reveal the temperature at which the carbonate formed. Certain clays indicate aridity.

Each material provides a proxy for what the environment was like during the formation of a sediment layer. Individually, however, the proxies and layers “are like blind men feeling the elephant,” Potts says. Only by combining them

can researchers assemble a cohesive picture. And that depends on accurately dating the sediments. At this point, the team isn’t even sure that the core stretches to the sought-after 500,000 year time point. Dating is Deino’s job, and that’s why at this party he is the guest of honor. If he can find volcanic material, he can use radiometric dating to determine when it formed, and so provide a chronology for the cores, pegging the ups and downs of each climatic indicator to actual dates.

A beefy, taciturn geochronologist at the Berkeley Geochronology Center in California, Deino commands a dedi-

icated computer terminal and one of the few stools in the lab—almost everyone else has to stand. He also gets first dibs on one-half of each sliced core. (The other halves are archived virtually untouched.) The cutaways reveal a panoply of colors and textures, ranging from midnight black to sunrise yellow, from fine clay grains to small pebbles. In some sections the layered striations are so narrow that they are barely discernible by the naked eye. >>



Assembly line. In a week, Richard Potts, Anna Behrensmeyer, and their colleagues studied all the core’s sections.



Behrensmeier studies enlarged photographs of the core for irregularities such as animal burrows or pieces of pumice, then pulls a dental pick from her hip pouch to tap the soil at those spots. Where the core seems to change character, René Dommain, a paleoecologist from the University of Greifswald in Germany, dissolves a tiny speck onto a slide and examines it with a petrographic microscope, whose polarizing filters help reveal the min-

from climate models and from marine sediments collected far from fossil sites. On the African continent, many local factors may have modified climate, such as rain shadows created by mountains or differences in altitude. Some basins may have been buffered from climate change and served as refugia.

The few cases where fossils are paired with local climate data offer tantalizing hints of support for Potts's idea. For example, layers of ancient sediment from outcrops in the Tugen Hills in Kenya reveal signs of wet and dry cycles, according to work by Yale's Hill and geologist John Kingston of Emory University in Atlanta. They collected diatoms, siliceous blue-green algae that grow in fresh water but vanish from sediments when a lake dries up. These tiny tracers mark when ancient lakes were full or dry, and showed that the Baringo Basin repeatedly filled and emptied every 20,000 years between 2.58 million and 2.69 million years ago. This was a response to cyclical changes in the orientation of Earth's axis as it orbits around the sun, the Milankovitch cycles, which in Africa create alternating wet and dry conditions. These cycles are the "pace-makers of African climate," deMenocal says.

The repeated shifts had a "kaleidoscopic" effect on animals in the basin, breaking up communities as the lakes filled, then allowing new mixes of species to reassemble when the lakes shrank, says Hill, whose team noted these shifts in fossils. One species that was part of the mix was *Homo*: The oldest *Homo* fossil was found just above a layer with diatoms, dating to 2.5 million years ago, suggesting that our genus arose just after the wet and dry cycles intensified in the Baringo Basin (*Science*, 4 February 2011, p. 540).

To nail down such links, however, researchers need local evidence, for example that climate changes preceded bursts of speciation, as predicted by the turnover-pulse hypothesis. The climate record near fossil outcrops is often incomplete. However, fine-scale evidence of such changes can come from the sediments that piled up

in the deepest part of ancient lakes, which trap chemical isotopes, pollen grains, charcoal, and other bits of detritus that offer telltale signs of past climates. "These lake sediments are like a metronome. They're accumulating all of the time," says paleolimnologist Andrew Cohen of the University of Arizona in Tucson. Adds Feibel: "Getting a lake record of environmental variability literally a few kilometers from the actual [hominin fossil] sites" can offer "an unprecedented perspective on when and how environmental fluctuations actually impacted these landscapes and habitats."

That's why this year, after 8 years of planning, an international team is drilling holes in six ancient lakebeds in Kenya and Ethiopia, says Cohen, who directs the \$5 million Hominin Sites and Paleolakes Drilling Project. In June, the group drilled down 228 meters and extracted cores in the Baringo Basin, hoping to sync the local wet-dry cycles with records from deep-sea cores, and to see local changes as early as 3.4 million years ago. Farther north, in the Awash valley, cores will test whether dry climate preceded the turnover in grazers 3 million years ago. If the cores don't show that climate changes intensified, the turnover-pulse hypothesis would be falsified in that lake basin. Conversely, if signs of grasses are common, the data may strengthen the revised savanna hypothesis.

The same drill rig is also taking cores from the habitats of other ancient hominins, including the site where a 1.6 million-year-old skeleton of *H. erectus* was found in the Lake Turkana Basin. The team also will core two lake basins in Ethiopia and Kenya that cover the time span drilled at Olororgesailie—the past 500,000 years, when *H. sapiens* was born. "Each of these places will give us an interesting time slice through human evolution," Cohen says. The cores will enable researchers to "ask some of the most existential questions of our time," he says: "What it means to be human and what were the environmental constraints on why we are the way we are."

—ANN GIBBONS

eral composition of the smeared sediment.

The smear slides reveal little sign of life in the core's youngest, uppermost layers. Then a slide taken at 37 meters down is littered with diatoms, tiny algae with distinctive siliceous shells that grow in specific environments. "We have a lake," Owen announces to the group; they erupt into a cheer. Potts and Behrensmeyer now know that their predictions are correct.

In subsequent slides, changes in the mix of diatoms reveal an ever-more saline environment as the

sediments get older. At about 44 meters down, diatoms peter out, perhaps indicating that the lake was temporary or too saline for diatoms.

Dommain also records sightings of fossilized fungal spores, bits of other algae, phytoliths, charcoal, and pollen, all of which can record environmental dynamics. The presence of one colonial alga, *Botryococcus*, for example, indi-

cates a shallow lake. Charcoal speaks to fires: The bigger the piece, the closer the fire was to the lake. "All this [variability] tells you it's not a stable environment," Dommain says.

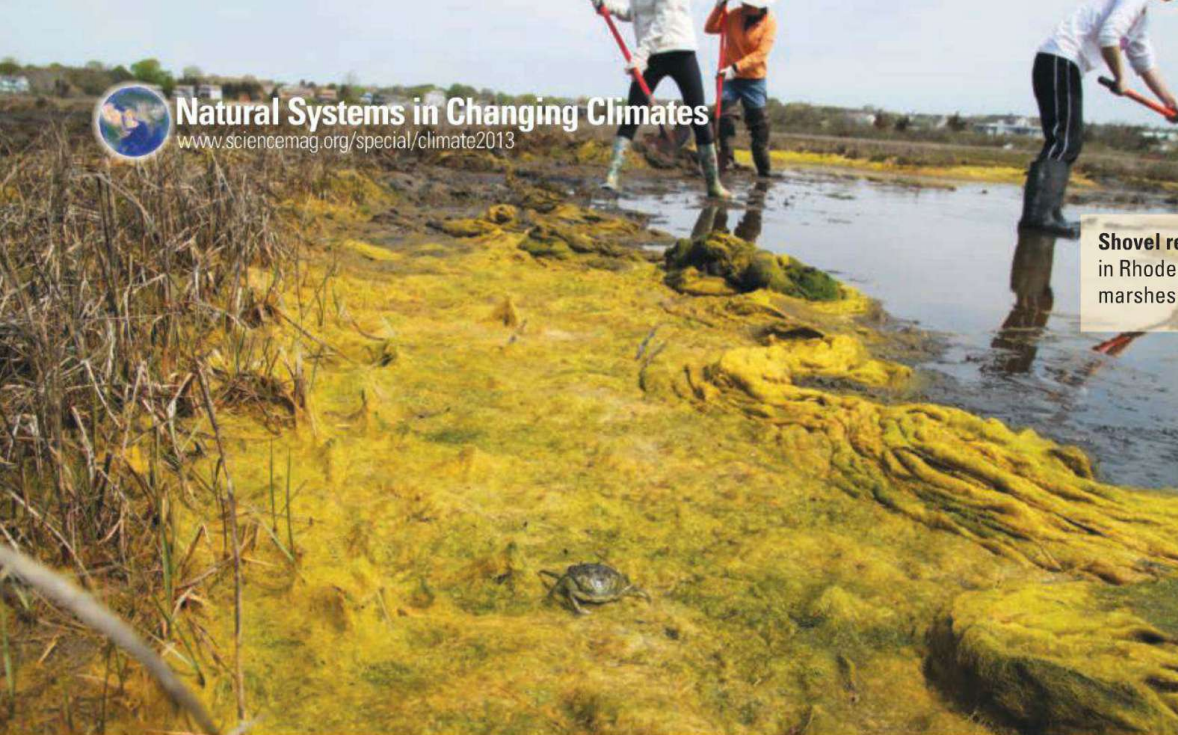
When the weeklong party finally breaks up, there is still plenty of work left. Over the next 18 months, the guests plan to complete their initial analyses and tie what they find to what they know from the outcrops. Ultimately, they'll develop a catalog of how the climate indicators changed over time, then attempt to model how climate changes affected the local ecosystem and the hominins who lived there. "We will have lots of stories" to tell, predicts Naomi Levin, a paleoecologist at Johns Hopkins University in Baltimore, Maryland. "But to figure out the importance for human evolution will take a lot more time."

—ELIZABETH PENNISI



Ready and waiting. At the LacCore lab in Minneapolis, 1.5-meter-long sections of core await processing.

CREDIT: JENNIFER CLARK



Shovel ready. For conservationists in Rhode Island, restoring coastal marshes requires boots on the ground.

Wet benefit

Although they're not the most glamorous biomes, the United Nations estimates that wetlands are one of the world's most valuable providers of "ecosystem services," such as storm protection, water filtering, and seafood production. They also help lock up as much as 450 billion metric tons of carbon globally, absorbing warming compounds that might otherwise leak into the atmosphere.

Marshes have already experienced centuries of

WETLANDS

Can Coastal Marshes Rise Above It All?

As climate change causes sea level to rise, wetland scientists are struggling to predict which salt marshes will drown—and which might climb out of danger

WESTERLY, RHODE ISLAND—Biologist Marci Cole Ekberg plunges her shovel into a particularly gloppy spot in a mucky salt marsh near the Atlantic Ocean. Her goal: to drain one of many shallow pools that are creating dead zones in the expanse of otherwise dense grasses, a phenomenon that she's recently observed in more than a dozen other marshes around the state. She fears that the pools are an early consequence of the sea-level rise that is being driven by global warming and an ominous "glimpse of the future" for marshes in New England. Rising oceans will drown the grasses, she worries, eliminating rich habitats and leaving coastlines bare.

Other researchers, however, are skeptical that the pockmarks are a result of climate change, saying winter ice or other causes may be to blame. And Rhode Island isn't the only place where researchers are debating what is really happening in salt marshes today and how the wetlands will fare in a future of higher seas. There's wide agreement that these salt marshes are among the ecosystems most vulnerable to rapid sea-level rise. But few researchers are ready to predict the fate of specific marshes; there's still too much to learn, they say, about how wetlands in different regions accumulate sediments that might allow them to outclimb rising waters and whether they can escape by migrating inland.

Wetlands scientists are mobilizing to reduce the uncertainty. By building improved forecasting models and better monitoring systems—and studying wetland regions already experiencing dramatic sea-level rise—they're hoping to bring some clarity to a murky topic and identify practical steps to protect marshes. The overarching goal, says wetlands researcher Susan Adamowicz of the U.S. Fish and Wildlife Service in Wells, Maine, is to help managers "give marshes the best possible chance to outpace global sea-level rise."

insults—such as pollution, overfishing, and draining for farming and development—that have disrupted the ecological systems that help keep them healthy. Now, rising temperatures are causing land-based ice sheets to melt and seawater to expand. Such changes have already helped push sea level up by an average of 1.4 to 3.7 millimeters per year since 1950, according to a 2010 study published in *Science*. (Other estimates vary.) Climate models predict that the trend will accelerate to 1 centimeter or more per year as Earth continues to warm. And even a few extra centimeters of water can mean the difference between life and drowning for marshes, which typically occupy a narrow coastal band that ends just above the high tide line.

Faced with rising water, marshes have three options, says geologist Matthew Kirwan of the U.S. Geological Survey (USGS) in Charlottesville, Virginia: build in place by trapping and piling up new sediments, migrate to higher ground inland, or die. Predicting which path a marsh might take, however, requires understanding the interplay of a host of factors, including the biological traits of different marsh grasses and how wetlands construct muddy yet firm foundations from grains of sand, silt, and organic litter.

A sinking laboratory

To get a glimpse of how these factors might shape marsh adaptability in the future, researchers have begun to scrutinize one wetland ecosystem already experiencing local sea-level rise: Louisiana's Mississippi delta along the Gulf of Mexico. There, natural and human factors are causing the land to sink relatively quickly, creating a natural laboratory that simulates a sea-level rise of 1 to 2 cm per year. That could be "what it's going to be like everywhere by the end of the century," says ecologist James Morris of the University in South Carolina, Columbia.

Some delta marshes are adapting better than others: While grasses in a spot named Old Oyster Bayou have thrived, for instance, those in nearby Bayou Chitique have been largely submerged. The difference, researchers say, highlights the important role that an adequate sup-

ply of fresh sediment can play in marsh survival. While Old Oyster Bayou receives some 70 mg of fresh sediment per liter of river water, allowing it to outclimb rising Gulf waters, Bayou Chitiqué's sediment infusions are largely blocked by upstream levees, reducing the load to just 20 mg per liter. The "natural process has been interrupted and there's not enough sediment," Morris says.

A 2010 modeling study that Kirwan and his USGS colleagues published in *Geophysical Research Letters* underscored the importance of sediment supply. In a scenario that included a rapid global sea-level rise of 1.25 m by 2100, the outlook for the 21st century was grim: "Most coastal wetlands worldwide will disappear," they concluded. But under slower scenarios, there was hope. Although marshes with low sediment availability fared poorly in the models, those with ample supplies often survived. A marsh's tidal range also played a role, the study found, with wetlands located in regions with larger gaps between low and high tide better situated to ride out sea-level rise, apparently because plants adapted for higher tidal ranges better withstand drowning.

Trench warfare

For conservationists, such studies suggest that it might be possible to help threatened wetlands adapt—for instance, by removing levees or dams to restore sediment, or even pumping in new mud. And in Rhode Island, the idea of ultimately aiding drowning marshes is what motivated Cole Ekberg, a biologist with the conservation group Save The Bay, to recently lug a shovel into a marsh here that is pockmarked with shallow grassless pools.

The origins and meaning of the pools is the subject of local debate, some fierce. Cole Ekberg and others say that their spread is a relatively recent development, documented in just the last few years in the higher-elevation parts of marshes in Rhode Island, Connecticut, Massachusetts, and Maine. And she's been running a restoration experiment of sorts, draining the pools to see if the grasses come back. "It's the best part of the day when water begins to move," she says.

Other marsh researchers are skeptical, blaming winter ice damage, invasive weeds, or geology. Mark Bertness, a marine ecologist at Brown University, sees "no evidence" of sea-level rise in the pools and says that the Save The Bay staff members are "well-intentioned but naïve."

Bertness also wonders whether the focus on sea-level rise is diverting attention from more immediate threats. His own studies, for instance, have shown that overfishing has resulted in a boom in a population of crabs that chow on marsh grass, sometimes causing severe damage. "I was just dumbfounded what these crabs have done over a 2, 3-year period," he says. "Sea-level rise is going to come along, but this is happening now."

No escape route

All sides, however, appear to agree that if a marsh doesn't have a sediment source that will allow it to build up, "then the question becomes will it be able to migrate," Kirwan says.

Increasingly, the answer is no. Marshes around the world are hemmed in by development that essentially blocks migration

to higher ground. In many areas, the obstacles are concrete or stone sea walls built to protect seaside homes or industrial sites. In Europe and parts of Asia, studies have found that two-thirds or more of many shorelines have been "armored." Even sparsely populated sites can leave marshes little room: A 2000 study of Maine's lightly inhabited Casco Bay found that one-fifth of its shoreline was armored.

Some researchers are beginning to look at ways to clear such obstacles. Around the Blackwater National Wildlife Refuge near Maryland's Chesapeake Bay, for instance, a coalition of conservation and government groups has embarked on an ambitious effort to identify potential obstacles and protect possible migration paths. The group is even eyeing pine forests and farm fields that may have the right topography and soil types to be converted to future marshes. The Nature Conservancy has launched a similar effort on Long Island in New York state, while Rhode Island officials, scientists, and activists are working on a statewide assessment to map out risks to wetlands under different scenarios.

It could take decades to realize such forward-thinking efforts, planners say. In the meantime, scientists say that they need better ways to monitor how marshes are doing now. A good start, a team of USGS researchers argued in an April paper in *Nature Climate Change*, would be to create a global

Bayou blues. Louisiana's disappearing marshes offer a glimpse of how global wetlands may respond to rising seas.



network of 14,000 relatively simple devices called surface elevation table markers. Secured to the ground beneath marshes, mangroves, and wetlands, they can register changes in the height of the marsh surface to an accuracy of 0.01 cm, more precise than surveys, LiDAR, or satellite readings. The authors say the network, which might cost \$8 million to create, "would allow policymakers to prioritize wetland sites for intervention."

That's a goal that Save The Bay's Cole Ekberg supports. "Someone might ask what's the point of protecting salt marshes anyway, if they're doomed in the long run," she says. "My answer is if we can extend their lives 20 or 30 years, it's a valuable investment."

—ELI KINTISCH



GEOGRAPHY

Worth a Thousand Words

Models and experiments only go so far in assessing the effects of climate change. For a reality check, researchers turn to historical photos

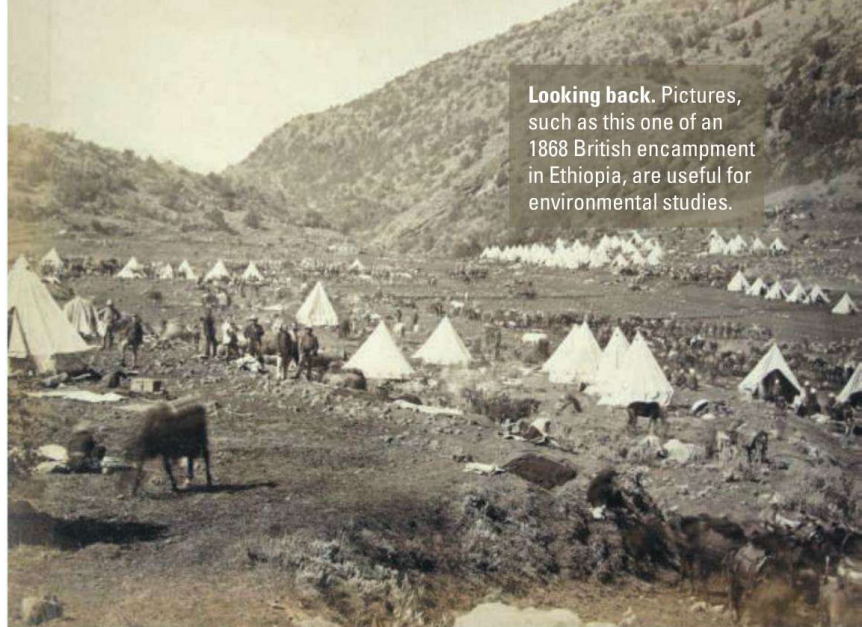
In 1868, British soldiers lugged a 500-kilogram camera into the Ethiopian mountains—not to shoot snapshots, but to photocopy documents for headquarters. Occasionally, however, they trained the lens on their surroundings. Now, 145 years later, the antique photos provide a unique window into how climate change and other factors have affected Ethiopian ecosystems. By comparing the historic images with modern photos snapped at the very same spots, researchers are documenting biological shifts that might be otherwise invisible. And Ethiopia isn't the only locale captured in historic photographs: Researchers have also turned up valuable troves from China and the Arctic. The photographs represent “a very powerful ecological tool,” says Isla Myers-Smith, an ecologist at the University of Edinburgh in the United Kingdom.

Although repeat photographs of receding glaciers yield perhaps the most iconic images of climate change, before-and-after images also

document more subtle biological shifts. Changes in forest or desert cover, acceleration of plant growth, and shifts in species can all show up. Realizing the potential of repeat photography, however, isn't easy.

First, researchers must track down potentially useful historic images. Most photos dating back a century or more were taken for reasons other than documenting the environment, so just a handful may be relevant to, for instance, climate change. “You can do a lot of looking,” Myers-Smith says. Then, scientists need to find out where the picture was taken and make the effort to return to the site. That may mean “hours of walking around looking for the ‘right’ bend in the stream or bump on the ridge,” she says.

But the effort can be worth it. Photo comparisons have yielded numerous insights and a few surprises. In these pages, *Science* takes a look at a few projects that use photos to go back in time.



Looking back. Pictures, such as this one of an 1868 British encampment in Ethiopia, are useful for environmental studies.

Advancing Seasons in China

Repeat photography was a labor of love for Yin Kaipu. In 2004, at age 60, the botanist from the Chengdu Institute of Biology in China, decided to follow in the footsteps of American plant collector Ernest H. Wilson. At the turn of the 20th century, camera in tow, Wilson explored western China for Harvard University's Arnold Arboretum. Much later, Yin covered some of the same territory for his work and was excited to recognize places where Wilson had taken photographs. “The landscape that I saw had already gone through great change since Wilson's time,” Yin says.

It took him 7 years to gather 1000 of the historical photos, 400 of which had potentially telltale landmarks. Based on books that traced Wilson's travel routes, Yin plotted a course through the 753,300 square kilometers of Sichuan, Hubei, and Chongqing provinces. He estimated that, in advance, he could pinpoint each location to within 30 kilometers.

Six years later—after raising \$200,000; traveling on foot, horseback, motorcycle, and boat; and pushing his body to its limits—



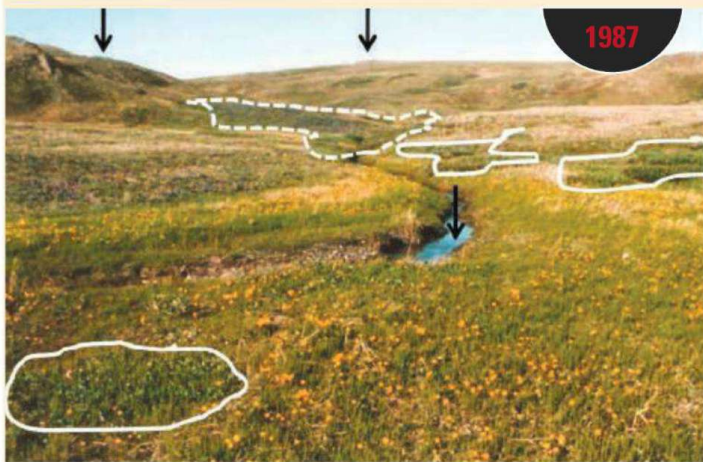
Yin had documented severe deterioration of the natural environment and a reduction in biodiversity, he says. The photos revealed climate change impacts as well. In one county, for instance, farmers plant rice a month earlier than they did 100 years ago. Elsewhere, the dates on the early and recent images showed

that a spring flower, *Primula*, also blooms a month early.

Yin is not the only repeat photographer combing China's landscapes. Conservation biologist Robert Moseley was exposed to the technique as an undergraduate in the 1970s, during a summer job in Idaho documenting

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1910
CREDITS (TOP TO BOTTOM): KING'S OWN ROYAL REGIMENT MUSEUM/WWW.KINGSCROWN.MUSEUM.PLUS.COM; ARNOLD ARBORETUM AND FELLOWS OF HARVARD COLLEGE/ARNOLD ARBORETUM ARCHIVES



1987



2009

Tundra in Turmoil

The new norm for the Arctic will not be vast expanses of frozen tundra and bare ground. Instead, warming temperatures have encouraged shrubs to sprout, and some of the first telltale signs of this change came from repeat photography. By 1996, satellite images were detecting more vegetation in the Far North, but it was a 2001 *Nature* paper that drove home what was happening. Matthew Sturm from the

U.S. Army Cold Regions Research and Engineering Laboratory in Fort Wainwright, Alaska, had rescued an impressive series of large-format photographs taken in the 1940s from low-flying aircraft during oil and natural resource surveys. The negatives, 46 centimeters by 23 centimeters, captured the landscape in exquisite detail, down to individual bushes. Sturm set out by helicopter to retake those photographs more than 50 years later. "We didn't know what we would find," recalls Ken Tape, an

Shrub invasion. In just 20 years, shrubs expanded on this meadow (white lines).

ecologist at the University of Alaska, Fairbanks, who worked with Sturm.

They ended up with nearly 300 pairs of photos. To date, they show that in 87% of the scenes, green alder and to a lesser extent dwarf birch and willow have grown bigger, filled in, or moved into previously shrub-free areas. Overall, shrub cover mush- >>



2007

Early bloomers. In 1910, Chinese farmers were just planting rice in June; by 2007, fields are quite far along by that time of year.

no data." For example, imagery for the Dry Valleys, a desertlike landscape, indicated that croplands have not expanded as researchers had thought, despite increases in population. The pictures also warn against a current practice of replacing grasslands with forests, as the early photos showed grasslands were the norm and suggested the environment is really better suited to grasslands, Moseley and his colleagues discovered. Both Yin's and Moseley's photos have also revealed nuanced changes to forest cover, rather than a unidirectional trend of forest loss as many had thought.

Moseley is no longer in China, but others, including the Chinese Academy of Sciences, are following up with similar studies. "I'm passionate about using this technique to inform conservation," Moseley says. "In places like Africa and parts of Asia, where good scientific data do not exist, photographs are a good way to get a quick historical profile."

how vegetation recovered from grazing. When he moved to the Tibet-Yunnan border for The Nature Conservancy in 2000, he turned to the method again, inspired by a magazine article with photographs taken in 1923 by *National Geographic* explorer Joseph Rock. With help from the National Geographic and Royal Geo-

graphical societies, he amassed 1100 photos taken before 1949 by 42 photographers, and he found and returned to 420 locations.

The paired photographs showed "patterns of environmental change and cultural land use that did not agree with the conventional wisdom," he says. "It turns out [the experts] had



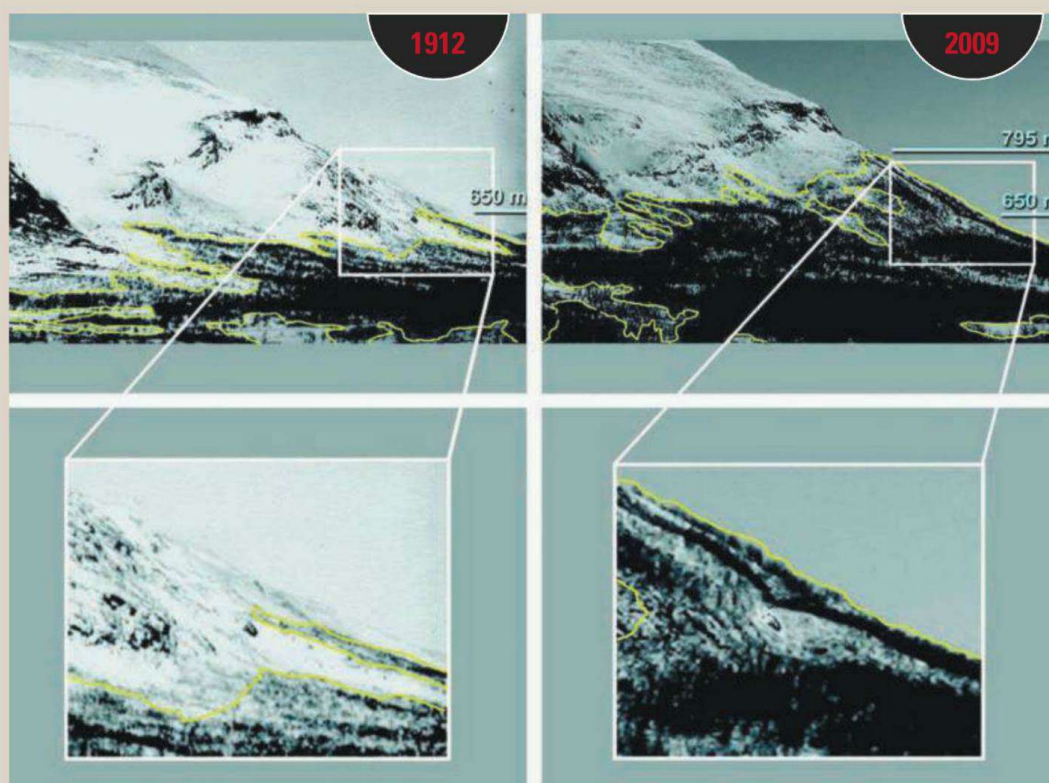
>> roomed by about 38%; only two photos showed a decline. “We can jump back 60 years and we definitely see widespread changes,” says Pieter Beck, an ecologist from the Woods Hole Research Center in Falmouth, Massachusetts.

Isla Myers-Smith of the University of Edinburgh in the United Kingdom was able to leap back even further in her studies of Arctic shrubs. On Herschel Island, located on the Arctic Coast of the Yukon Territory, she and her colleagues found photo albums dating back more than a

century chronicling the island’s use as a whaling station, police post, and mission. About 50 images showed plots of tundra, five of which she was able to locate again for repeat photographs. All five pairs showed more shrubs, she and her colleagues reported in 2011. More recent comparisons show that shrub expansion is continuing and can be documented over just 20 years.

Other researchers, meanwhile, have seen similar changes in more than two dozen locales

across the Arctic. Now, Myers-Smith, Tape, and Beck belong to a consortium called Shrub Hub that combines repeat photography with vegetation surveys, satellite monitoring, tree ring growth studies, and other work to better understand why shrubs are proliferating in the Arctic. “More shrubs could change carbon stores in the soil, habitat for caribou and other wildlife, and even how the tundra reflects the sun’s heat when the shrubs stick up above the snow,” further fueling warming, she notes.



Forest in motion. In Sweden, trees moved up a mountain slope, but climate change was not the reason, removal of human impact was.

Tree Line Shifts

Repeat photography is helping reveal when a warmer climate is affecting vegetation—and when it isn’t.

In the American Southwest, work by Robert Webb, a plant ecologist recently retired from the U.S. Geological Survey in Tucson, Arizona, has shown that fewer, shorter freezes are paving the way for more cactus seedlings and certain frost-sensitive shrubs to survive winters. Among Webb’s 10,000 paired photographs—some spanning 140 years—are many showing increases in these desert plants.

In subarctic Sweden, however, some paired photos tell a different story. Modeling predicts that in Sweden’s mountainous highlands, milder conditions will enable alpine forests to climb up slopes, as harsh higher altitudes become habitable for seedlings.

To see if the forest was on the move, Rik Van Bogaert, a biogeographer from Ghent University in Belgium, compared photos of eight mountain scenes taken over a century. Some of the photos were taken around 1910 to document the newly built railroad, others were taken in the 1950s by a master’s student studying tree lines, and a few by Van

Bogaert himself in 2009.

About half of the images revealed climbing tree lines. But additional historical documents, as well as tree-ring analyses, indicated that warming conditions are not the main cause. Instead, the trees appear to be gradually reclaiming areas that reindeer herders had used for grazing and camping into the 1920s.

Such findings have made Van Bogaert a big fan of historical photos. “Not many methodologies,” he notes, allow researchers “to study ecosystem changes at a temporal scale of 100 years.”



Humans Greening A Landscape

The camera the British army sent to northern Ethiopia in 1868 captured 30 landscape photos that were of interest to Jan Nyssen, a geographer now at Ghent University in Belgium. They've proved unusually valuable, given the scant historical information about vegetation, terrain, and land use in the region.

All told, Nyssen has now amassed historical photos of Ethiopia from about 20 sources, spanning 1867 to the present. He's been able to create some 500 then-and-now pairs and has been surprised by some of the differences they show. With population increases and climate changes, he and others expected the landscape to become degraded and more desertlike over time. But many sites have more trees and shrubbery now than when the

Greening trend. A dry Ethiopian landscape has since sprouted more trees, thanks in part to conservation efforts.

British marched through this mountainous region, he says.

The trend toward a richer landscape was not consistent, however. The photos suggest an increase in vegetation through the 1930s, then a degrading landscape into the 1970s, with severe desertification between 1975 and 1984. Since then, there appears to have been a gradual improvement as the government and individual landowners have become more conservation and management-oriented. So far, Nyssen says, "most changes



are related to direct human intervention and that's overriding climate change." Still, the photos should have "relevance" for studying climate change, he adds, as global warming takes hold.

—ELIZABETH PENNISI

REVIEW

Changes in Ecologically Critical Terrestrial Climate Conditions

Noah S. Diffenbaugh^{1,2*} and Christopher B. Field³

Terrestrial ecosystems have encountered substantial warming over the past century, with temperatures increasing about twice as rapidly over land as over the oceans. Here, we review the likelihood of continued changes in terrestrial climate, including analyses of the Coupled Model Intercomparison Project global climate model ensemble. Inertia toward continued emissions creates potential 21st-century global warming that is comparable in magnitude to that of the largest global changes in the past 65 million years but is orders of magnitude more rapid. The rate of warming implies a velocity of climate change and required range shifts of up to several kilometers per year, raising the prospect of daunting challenges for ecosystems, especially in the context of extensive land use and degradation, changes in frequency and severity of extreme events, and interactions with other stresses.

Even before biogeographic relationships between vegetation and climate were described by von Humboldt (1), every traveler had the opportunity to see climatic controls on ecosystems expressed on every mountain range and across continents. Indeed, the earliest evidence for past climate changes came from mismatches between the current and fossil distributions of plants and animals. Some of the observed range shifts were hundreds or thousands of kilometers, a vagility that might be relevant to future migrations. But is it really? Individuals and species can potentially respond to changes in climate through a variety of pathways, including migration in space that allows persistence of the current climate conditions in a new geographic range and behavioral and/or evolutionary adaptations that allow persistence of the current geographic range in the face of new climate conditions (2). However, failure to respond sufficiently rapidly can result in species extinction (2, 3).

The sensitivity of plants, animals, and ecosystems to climate and climate-related processes is broadly documented (4–7). Evidence for this sensitivity arises from the patterns expressed on the current landscape; observations of recent, historical, and fossil range shifts; results of manipulative experiments; and inferences based on empirical and process models (2). However, despite this body of evidence, a number of complexities pose important challenges to impact assessment, including the dispersal ability of different taxa (8), the evolutionary response of

individual species (9, 10), the ecological response to novel climates (11–14), and the potential for climate “refugia” within the current geographic range (15). Further, species and ecosystems will encounter not only a range of climate conditions that is potentially different from any in the past but also the broader conditions of the Anthropocene (16), in which human actions either dominate or strongly influence a wide range of Earth system processes (17). The impacts of climate change will therefore result from interactions with other stresses, such as land use change, biological invasives, and air and water pollution (17–19). Recognizing the potential importance and limited understanding of physical climate changes; the behavioral, evolutionary, and ecological responses to those changes; and other interacting stresses can provide a starting point for managing evolving risks (20, 21).

Since the beginning of the 20th century, global mean temperature has increased by $\sim 0.8^\circ\text{C}$ and has been accompanied by rising sea level, altered seasonality, and changes in extremes (22). Since 1979, surface air temperatures over land have increased at about twice the rate of temperatures over oceans (23). It is very likely that warming will continue, with the magnitude determined by a combination of intrinsic features of the Earth system and human actions (22, 24).

Assessment of possible future changes in ecologically critical climate conditions requires three different kinds of information. First is an understanding of the aspects of climate change that drive biological response. Second is a comparison of current and future climate change with examples from the past, including both the magnitude and rate of change. Third is a picture of the context in which current climate change is occurring, and the consequences of that context in structuring constraints and opportunities. We

address all three elements, emphasizing the physical climate.

Projected Climate Change over the 21st Century

The trajectory of climate over the 21st century depends on three classes of factors: (i) the energy imbalance already built into the system as a result of past forcing by greenhouse gases (GHGs) and other changes (25); (ii) the intrinsic sensitivity of the climate system to anthropogenic forcing (26), including atmospheric, carbon-cycle, and other feedbacks (27); and (iii) the magnitude of future forcing, such as by GHGs and aerosols not yet released (28). Analyses of observed trends and geologic records provide critical insights for the first two kinds of factors, but uncertainties about the rate and pathway of future emissions create a need for controlled experiments that can account for potential thresholds, feedbacks, and nonlinearities. Because such experiments cannot be run on the real global system, climate models are used to explore possible futures.

Phase 5 of the Coupled Model Intercomparison Project (CMIP5) includes contributions from 25 modeling centers, using models with multiple structures, parameterizations, and realizations within a given forcing pathway (29). Climate forcings are provided by Representative Concentration Pathways (RCPs), which characterize the most important features of feasible alternative futures and are designed to be consistent with physical, demographic, economic, and social constraints (28, 30). The RCPs, like the Special Report on Emissions Scenarios (SRES) (31) and other earlier scenarios, are not intended as predictions and are not assigned probabilities or other indicators of expectation. Each RCP reaches a different level of anthropogenic radiative forcing in 2100, ranging from 2.6 W/m^2 for RCP2.6 to 8.5 W/m^2 for RCP8.5.

We discuss simulation results for the full range of RCPs, but with more examples from RCP8.5 because actual emissions since 2000 have been closest to RCP8.5 (32) and RCP8.5 spans the full range of 21st-century forcing encompassed by the RCPs (33). For the next few decades, when historical warming will be maintained by emissions that have already occurred (34–36) and when any investments in mitigation will still be building momentum, differences across the RCPs are small (33). The latter decades of the 21st century are really the era of climate options, in which differences in emissions—including in the near term—have potentially large consequences for climate.

For RCP8.5, the CMIP5 ensemble exhibits substantial warming over all terrestrial regions by the 2046–2065 period (Fig. 1) (37). The largest annual warming occurs over the Northern Hemisphere high latitudes, including $>4^\circ\text{C}$ above the 1986–2005 baseline (or about 5°C above pre-

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industrial temperatures) (Fig. 1) (22). Annual warming exceeds 2°C over most of the remaining land area in 2046–2065, including greater than 3°C over large areas of North America and Eurasia. By 2081–2100, warming exceeds 4°C over most land areas, with much of northern North America and northern Eurasia exceeding 6°C. The CMIP5 pattern of mean

warming is consistent between intermediate and high levels of forcing, as was the case with CMIP3 (38).

Substantial changes in annual precipitation emerge over some areas by 2046–2065 in RCP8.5, including increases over the high northern latitudes and decreases over the Mediterranean region and the mediterranean-climate regions of

southwestern South America, Africa, and Australia (Fig. 1). These patterns intensify by 2081–2100. The comparison between the 2046–2065 and 2081–2100 periods of RCP8.5 suggests the persistence of some regions that become drier and some that become wetter, with spatially durable patterns that increase in magnitude in response to increased forcing.

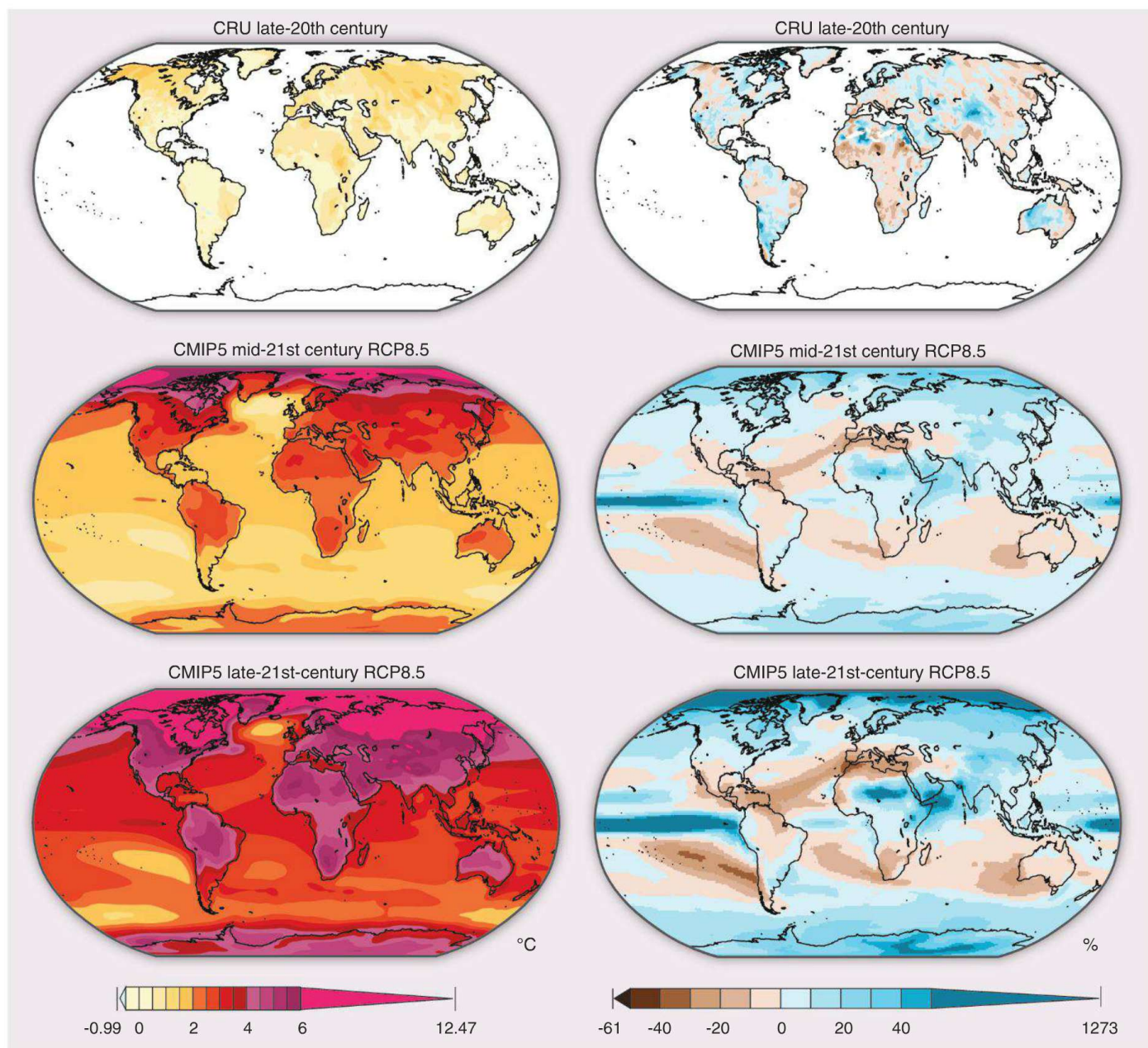


Fig. 1. Observed and projected changes in annual temperature and precipitation. (Top) Climatic Research Unit (CRU) observations (which are available only over land), calculated as 1986–2005 minus 1956–1975. (Middle) Differences in the mid-21st-century period of the CMIP5 RCP8.5 ensemble, calculated as 2046–2065 minus 1986–2005. (Bottom) Differences in the late-21st-century period of the CMIP5 RCP8.5 ensemble, calculated as 2081–2100 minus 1986–2005. We show the multi-model mean, using the model aggregation of Diffenbaugh and Giorgi (65). This presentation does not

indicate significant differences from background variability, nor does it reflect many other potentially important sources of uncertainty, including level of emissions, Earth system feedbacks, or model structure. The values at the left and right extremes of the color bars give the minimum and maximum values (respectively) that occur across all of the periods. The minimum temperature, minimum precipitation, and maximum precipitation extreme changes are all in the CRU observations. Further details are provided in the supplementary materials.

Climate Extremes

Sensitivity to climate extremes can be found in tropical, temperate, and boreal ecosystems. For example, tree mortality in the Amazon has been linked to drought (39–41), severe heat (42), and extreme wind (43). Drought and human-induced biomass burning and deforestation (44–46) combine to increase tropical forest fires—and loss of tropical forest cover—during strong El Niño events. Temperate ecosystems experience forest die-off (47–49) and decreased primary production (50) in response to severe heat and drought, with low spring and summer snowmelt runoff increasing stress on mountain, riparian, and dry-land ecosystems (51–54) through increased pest pressure (55), wildfires (52), and decreased water supply for riparian and montane ecosystems (51, 56, 57). In the Arctic, extreme winter warm events can cause vegetation damage and reduced summer growth (58), alteration of community composition (59), and changes in microbial habitats (including loss of ice and thawing of permafrost) (60), whereas drought and temperature stress can limit boreal forest growth and carbon uptake (61–63).

A large body of literature, assessed in the 2012 Intergovernmental Panel on Climate Change (IPCC) *Special Report on Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation* (64), indicates that further global warming is likely to alter the occurrence, severity, and/or spatial pattern of a number of different types of climate extremes. CMIP5 projects substantial increases in the occurrence of extreme hot seasons in both RCP4.5 and RCP8.5 (65), with most land areas experiencing >50% of years with mean summer temperature above the late-20th-century maximum by 2046–2065 in RCP8.5, and >80% of years by 2080–2099 (Fig. 2) (65). These same projections include increases in the frequency of extremely dry seasons by 2080–2099, with areas of Central America, northeastern South America, the Mediterranean, West Africa, southern Africa, and southwestern Australia all exhibiting >30% of years with mean seasonal precipitation below the late-20th-century minimum (Fig. 2) (65). The occurrence of extremely low spring snow accumulation is also projected to increase in

much of the Northern Hemisphere, including >80% of years below the baseline minimum over areas of western North America by 2080–2099 (Fig. 2) (66).

A number of daily-scale extremes are also projected to change in response to elevated GHG forcing (64). For RCP8.5, CMIP5 simulates statistically significant increases in the occurrence of daily-scale hot extremes over all land areas and in the occurrence of extreme daily-scale wet events over most land areas (excepting the areas of robust drying seen in our Fig. 1) (67). Climate model experiments also project the hydrologic intensity—as measured by the combination of daily-scale precipitation intensity and dry spell length—to increase over almost all land areas in response to continued global warming (68). Further, the occurrence of

frost days and severe cold events, which can be critical for limiting the ranges of a number of species [including some pests (69)], decrease in response to further global warming (67, 70, 71). The greatest uncertainties in daily-scale extremes are associated with severe storms such as tropical cyclones and tornadoes, which exhibit complex physical dynamics and incomplete observational records (72–76).

Some changes in extremes already have been observed (64). For example, the fraction of land area experiencing extreme seasonal heat has increased over the past three decades, both globally and over most tropical and some mid-latitude land regions (Fig. 2) (77). The intensity, occurrence, and duration of heat waves have likewise increased globally (78), whereas the occurrence of daily-scale cold extremes

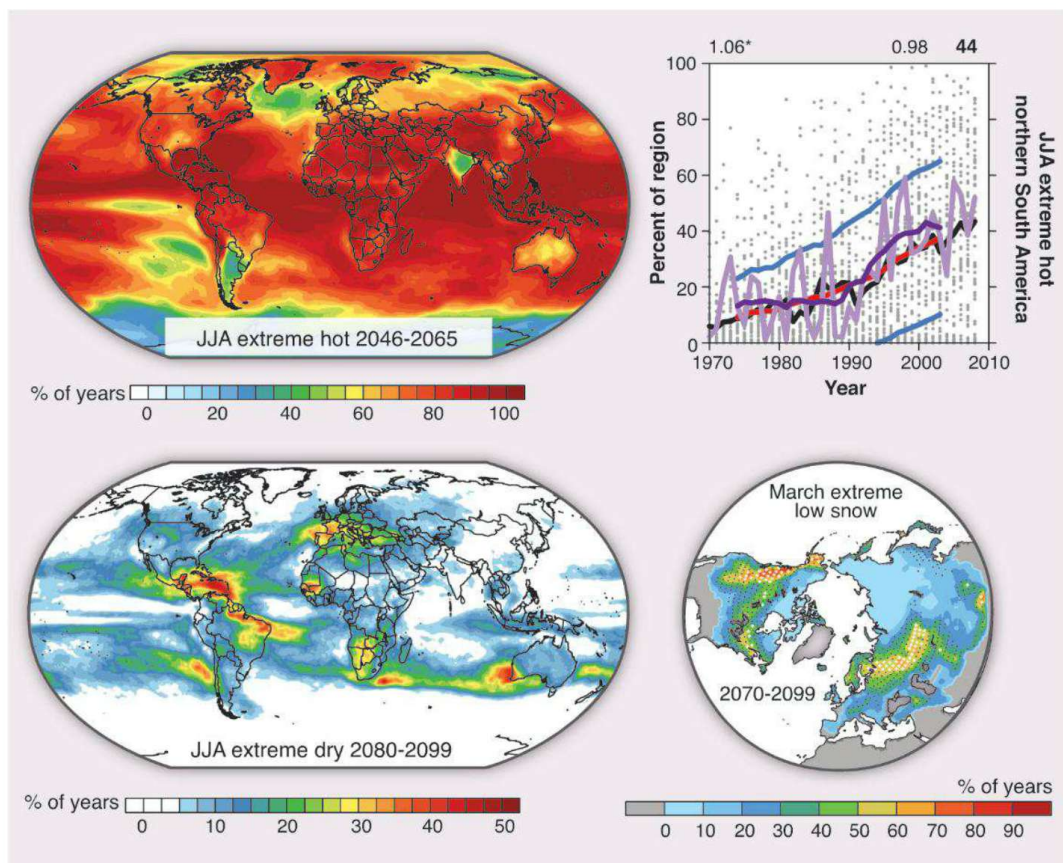


Fig. 2. Changes in seasonal extremes. (Left) The frequency of the 1986–2005 maximum June–July–August (JJA) temperature (top left) and minimum JJA precipitation (bottom left) in the 2046–2065 and 2080–2099 periods of RCP8.5 [from (65)]. (Bottom right) The frequency of the 1976–2005 minimum March snow water equivalent in the 2070–2099 period of RCP8.5, with black (white) stippling indicating areas where the multimodel mean exceeds 1.0 (2.0) SD of the multimodel spread [from (66)]. (Top right) The fraction of land grid points in northern South America with JJA surface air temperatures above the respective 1952–1969 maximum [from (77)]. The light and dark purple show the annual and 10-year running mean of the observational time series, with the trend shown in the top left (percent of region per year; asterisk indicates statistical significance). The gray points show each CMIP3 realization, the black and red show the annual and 10-year running mean, and the blue shows a 1-SD range. The mean of the trends in the CMIP3 realizations is shown in the top right, with the number of realizations (out of 52) that exhibit a statistically significant trend shown in bold. Further details are provided in the supplementary materials.

has decreased globally and over most extra-tropical land areas (79). The occurrence of extreme wet events has also increased globally (80), although not all regions exhibit uniformly increasing trends (81). Last, droughts have increased in length or intensity in some regions (64), and the hydrologic intensity has increased over many land areas (although the observed signal is less uniform than the simulated response to further global warming) (68).

A Range of Possible Futures

A key source of uncertainty for ecosystem impacts is the magnitude of climate change that ecosystems will encounter in the coming decades. Multiple factors contribute to this uncertainty, including the magnitude of global-scale feedbacks [such as from clouds (82) and the carbon cycle (83)], the response of certain extreme events to elevated forcing (72, 73), and the influence of internal climate variability on the local climate trend (84). The level of GHG emissions from human activities is, however, the largest source of uncertainty in the magnitude of global climate change on the century time scale (27, 85, 86), with uncertainties about physical climate mechanisms contributing a progressively larger fraction of uncertainty at smaller spatial and temporal scales (87).

The feasible range of human GHG emissions is very large (Fig. 3) (30, 88–94). The RCPs span a range from less than 450 parts per million (ppm) carbon dioxide (CO₂) for RCP2.6 to

greater than 925 ppm in 2100 for RCP8.5 (Fig. 3) (30). Although RCP2.6 is considered technically feasible, it requires economy-wide negative emissions in the second half of the 21st century, meaning that the sum of all human activities is a net removal of CO₂ from the atmosphere (95). On the other hand, a world in which all countries achieve an energy profile similar to that of the United States implies greater emissions than in RCP8.5 (88). Further, combustion of all remaining fossil fuels could lead to CO₂ concentrations on the order of 2000 ppm, with concentrations remaining over 1500 ppm for 1000 years (Fig. 3) (91).

Despite important uncertainties about the magnitude of future global warming, several sources of inertia make some future climate change a virtual certainty. Ocean thermal inertia causes global temperature to increase even after atmospheric CO₂ concentrations have stabilized (27, 35) and regional climate to change even after emissions have ceased and global temperature has stabilized (96). Carbon-cycle inertia and ocean thermal inertia cause global temperature to remain elevated long after emissions have stopped, even as CO₂ concentrations in the atmosphere decrease (34–36). If climate changes cause widespread forest loss and/or thawing of permafrost, substantial carbon input to the atmosphere could continue even after anthropogenic CO₂ emissions have ceased (97–99).

In addition to these physical, biogeochemical, and ecological sources of inertia, the human dimension of the climate system creates inertia that is likely to prolong and increase the level of global warming. The existing fossil-fuel-based economy creates inertia toward further CO₂ emissions. The life cycle of existing infrastructure and the knowledge base for generating wealth from fossil energy resources together imply that CO₂ emissions will continue for a minimum of another half-century (90). Human dynamics also create further inertia (92, 93). Increasing global population increases the global demand for energy, which in the current fossil-fuel-based energy system implies increasing global CO₂ emissions, even without economic development (88). However, demand for energy-enabled improvement in human well-being creates additional inertia (88), particularly given that 1.3 billion people currently lack reliable access to electricity, and 2.6 billion people rely on biomass for cooking (100). Last, the political process provides further inertia, both because emissions continue as political negotiations take place and because mitigation proposals are built around gradual emissions reductions that guarantee further emissions even if such proposals are eventually adopted (28, 101, 102).

Although not literally “committed,” these forms of inertia linked to human actions increase the likelihood that terrestrial ecosystems will face GHG concentrations that have rarely been encountered since the deep past. Ice core data confirm that atmospheric CO₂ concentrations have not been as high as at present for at least 800,000 years (Fig. 3) (103). Geochemical models and most proxy data also indicate CO₂ concentrations below 600 ppm—and, except for a small fraction of the record, below present levels—over the past 22 million years (Fig. 3) (104, 105). The trajectories of human population, energy demand, economic development, and climate policy therefore create the very real possibility that over the coming century, atmospheric CO₂ concentrations will be the highest of the past 22 million years (Fig. 3), with the trajectory of other GHGs further enhancing the total radiative forcing (30).

The Velocity of Climate Change

The rate of change in GHG concentrations and climate during the Anthropocene has been—and has the potential to continue to be—exceedingly rapid relative to past changes (Fig. 3 and fig. S1) (106–112). For example, although the global cooling that occurred between the early Eocene and the Eocene-Oligocene glaciation of Antarctica (52 to 34 million years ago) was greater than the 21st-century warming projected for RCP8.5, the Eocene cooling occurred over ~18 million years, making the rate of change many orders of magnitude slower than those of the RCPs

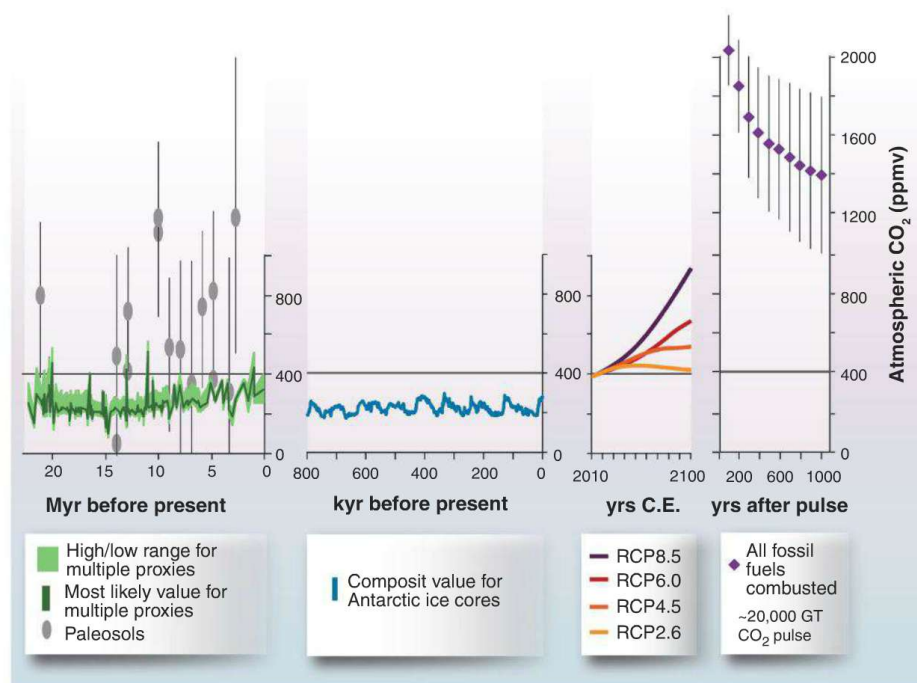


Fig. 3. Past and potential future atmospheric CO₂ concentrations. (Left) The high-low range of CO₂ over the past 22 million years from phytoplankton/forams, stomatal indices/ratios, and marine boron (105). (Middle left) CO₂ from Antarctic ice cores (103). (Middle right) CO₂ concentrations for different RCPs (30). (Right) The high-low range of CO₂ concentrations for the 1000-year time horizon after all fossil fuels are combusted (91). Further details are provided in the supplementary materials.

(fig. S1) (33, 112). Likewise, the Paleocene-Eocene Thermal Maximum (PETM) encompassed warming of at least 5°C in <10,000 years (113), a rate of change up to 100-fold slower than that projected for RCP8.5 and 10-fold slower than that projected for RCP2.6. Records from high-resolution ice cores indicate that regional climates can reorganize quickly, especially during glacial/interglacial transitions (114), but global rates of change during events such as the last glacial termination and the late-glacial/early-Holocene warming were all well below the minimum rate for the RCPs (fig. S1). Further, the rates of global change during the Medieval Climate Anomaly (MCA), Little Ice Age (LIA), and early Holocene were all smaller than the observed rates from 1880 to 2005 and than for the committed warming calculated to occur over the 21st century if atmospheric concentrations were capped at year-2000 levels (fig. S1).

The potentially unprecedented rate of global warming over the next century may present challenges for many terrestrial species as favorable climatic conditions shift rapidly across the landscape. Despite the fact that the tropics have exhibited the smallest absolute magnitude of warming (Fig. 1), the low background variability of annual and seasonal temperatures is causing temperature change to emerge most quickly from the background variability over the tropics (77, 115). In future decades, low-latitude warming (65, 77, 116) will likely expose many organisms in regions of high biodiversity and endemism to novel climate conditions, including frequent occurrence of unprecedented heat (Fig. 2) (116).

Another measure of potential climate stress is the velocity of climate change (8, 37), or the distance per unit of time that species need to move to keep conditions within the current local envelope (Fig. 4) (7, 8, 106, 108–110, 116–120). Different measures of velocity tend to emphasize either local topographic effects or large-scale climate patterns. Methods that understate the role of topography (Fig. 4) can miss the potential for the creation of climate refugia that could allow species to persist in the current range despite changes in large-scale climate conditions (15). Conversely, methods that underrepresent large-scale climate patterns ignore the critical fact that substantial changes can effectively push many species off the tops of mountains (121, 122) or the poleward edges of continents (Fig. 4). Moreover, biotic factors (8) such as evolutionary adaptation, dispersal ability, habitat suitability, and ecological interactions need also to be considered.

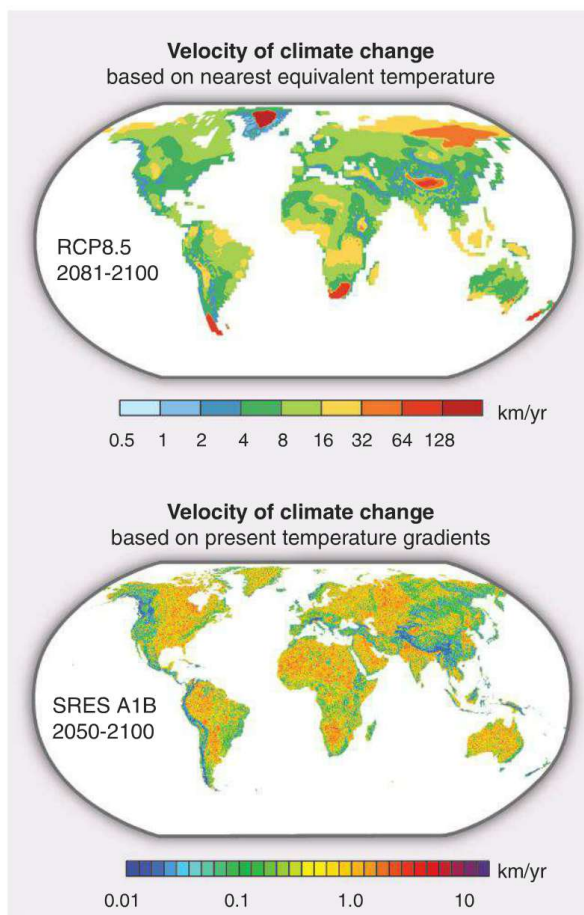


Fig. 4. The velocity of climate change. (Top) The climate change velocity in the CMIP5 RCP8.5 ensemble, calculated by identifying the closest location (to each grid point) with a future annual temperature that is similar to the baseline annual temperature. (Bottom) The climate change velocity [from (117)], calculated by using the present temperature gradient at each location and the trend in temperature projected by the CMIP3 ensemble in the SRES A1B scenario. The two panels use different color scales. Further details are provided in the supplementary materials.

The velocity of climate change may present daunting challenges for terrestrial organisms (7, 8, 69, 118, 123–129). Much of the world could experience climate change velocities greater than 1 km/year over the 21st century, and in some locations, the velocities could be much higher (Fig. 4) (8, 119). A rapidly increasing body of work (8, 11, 120, 129) has evaluated the dispersal potential of individual species in the context of expected velocities of climate change. Many species have the potential to keep pace with the shifting climate (8, 11, 120), but ability may or may not predict success. In some cases, the constraint may be no-analog climates, in which altered relationships between temperature and precipitation or novel patterns of extremes greatly restrict suitable habitat (14). In other cases, the limiting (or enhancing) factors may be the alteration of important biotic in-

teractions, the ability of existing species to hold onto habitat, or the presence of invasives that can quickly colonize and dominate available sites (11, 130). And in many locations, the constraint will be habitat fragmentation or degradation resulting from land use or air or water pollution (8, 131).

Conclusions

Terrestrial ecosystems have experienced widespread changes in climate over the past century. It is highly likely that those changes will intensify in the coming decades, unfolding at a rate that is at least an order of magnitude—and potentially several orders of magnitude—more rapid than the changes to which terrestrial ecosystems have been exposed during the past 65 million years. In responding to those rapid changes in climate, organisms will encounter a highly fragmented landscape that is dominated by a broad range of human influences. The combination of high climate-change velocity and multi-dimensional human fragmentation will present terrestrial ecosystems with an environment that is unprecedented in recent evolutionary history.

However, the ultimate velocity of climate change is not yet determined. Although many Earth system feedbacks are uncertain, the greatest sources of uncertainty—and greatest opportunities for modifying the trajectory of change—lie in the human dimension. As a result, the rate and magnitude of climate change ultimately experienced by terrestrial ecosystems will be mostly determined by the human decisions, innovations, and economic developments that will determine the pathway of GHG emissions.

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Supplementary Materials

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Materials and Methods

Fig. S1

Table S1

References (132–135)

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REVIEW

Marine Ecosystem Responses to Cenozoic Global Change

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The future impacts of anthropogenic global change on marine ecosystems are highly uncertain, but insights can be gained from past intervals of high atmospheric carbon dioxide partial pressure. The long-term geological record reveals an early Cenozoic warm climate that supported smaller polar ecosystems, few coral-algal reefs, expanded shallow-water platforms, longer food chains with less energy for top predators, and a less oxygenated ocean than today. The closest analogs for our likely future are climate transients, 10,000 to 200,000 years in duration, that occurred during the long early Cenozoic interval of elevated warmth. Although the future ocean will begin to resemble the past greenhouse world, it will retain elements of the present “icehouse” world long into the future. Changing temperatures and ocean acidification, together with rising sea level and shifts in ocean productivity, will keep marine ecosystems in a state of continuous change for 100,000 years.

Marine ecosystems are already changing in response to the multifarious impacts of humanity on the living Earth system (1, 2), but these impacts are merely a prelude to what may occur over the next few millennia (3–9). If we are to have confidence in projecting how marine ecosystems will respond in the future, we need a mechanistic understanding of Earth system interactions over the full 100,000-year time scale of the removal of excess CO₂ from the atmosphere (10). It is for this reason that the marine fossil record holds the key to understanding our future oceans (Fig. 1). Here, we review the marine Cenozoic record [0 to 66 million years ago (Ma)], contrast it with scenarios for future oceanic environmental change, and assess the implications for the response of ecosystems.

In discussions of the geologic record of global change, it is important to distinguish between

mean and transient states. Mean climate states consist of the web of abiotic and biotic interactions that develop over tens of thousands to millions of years and incorporate slowly evolving parts of Earth's climate, ocean circulation, and tectonics. Transient states, in comparison, are relatively short intervals of abrupt (century- to millennium-scale) climate change, whose dynamics are contingent on the leads and lags in interactions among life, biogeochemical cycles, ice growth and decay, and other aspects of Earth system dynamics. Ecosystems exhibit a range in response rates: Animal migration pathways and ocean productivity may respond rapidly to climate forcing, whereas a change in sea level may reset growth of a marsh (11) or sandy bottoms on a continental shelf (12) for thousands of years before these ecosystems reach a new dynamic equilibrium. Thus, both mean and transient dynamics are important for understanding past and future marine ecosystems (13).

Past Mean States: The Cenozoic

The evolution of marine ecosystems through the Cenozoic can be loosely divided into those of the “greenhouse” world (~34 to 66 Ma) and those

of the modern “icehouse” world (0 to 34 Ma) (Fig. 1). We explore what these alternative mean states were like in terms of physical conditions and ecosystem structure and function.

Greenhouse World Physical Conditions

Multiple lines of proxy evidence suggest that atmospheric partial pressure of CO₂ (*p*CO₂) reached concentrations above 800 parts per million by volume (ppmv) between 34 and 50 Ma (14) (Fig. 2). Tropical sea surface temperatures (SSTs) reached as high as 30° to 34°C between 45 and 55 Ma (Fig. 2). The poles were unusually warm, with above-freezing winter polar temperatures and no large polar ice sheets (15, 16). Because most deep water is formed by the sinking of polar surface water, the deep ocean was considerably warmer than now, with temperatures of 8° to 12°C during the Early Eocene (~50 Ma) versus 1° to 3°C in the modern ocean (15). The lack of water storage in large polar ice sheets caused sea level to be ~50 m higher than the modern ocean, creating extensive shallow-water platforms (15, 17).

In the warm Early Eocene (~50 Ma), tectonic connections between Antarctica and both Australia and South America allowed warm subtropical waters to extend much closer to the Antarctic coastline, helping to prevent the formation of an extensive Antarctic ice cap (16) and limiting the extent of ocean mixing and nutrient delivery to plankton communities in the Southern Ocean (18). Tectonic barriers and a strong poleward storm track maintained the Arctic Ocean as a marine anoxic “lake” with a brackish surface-water lens over a poorly ventilated marine water column (19). Indeed, the Arctic surface ocean was occasionally dominated by the freshwater fern *Azolla*, indicating substantial freshwater runoff (20).

Greenhouse World Ecosystems

The warm oceans of the early Paleogene likely supported unusual pelagic ecosystems from a modern perspective. The warm Eocene saw oligotrophic open-ocean ecosystems that extended to the mid- and high latitudes and productive equatorial zones that extended into what is now the

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warm subtropics (17). In the modern ocean, most primary productivity in warm, low-latitude gyres is generated by small phytoplankton with highly

efficient recycling of organic matter and nutrients (21). Such picophytoplankton-dominated ecosystems typically support long food chains

where the loss of energy between trophic levels limits the overall size of top predator populations (5, 22). Although the radiations of many

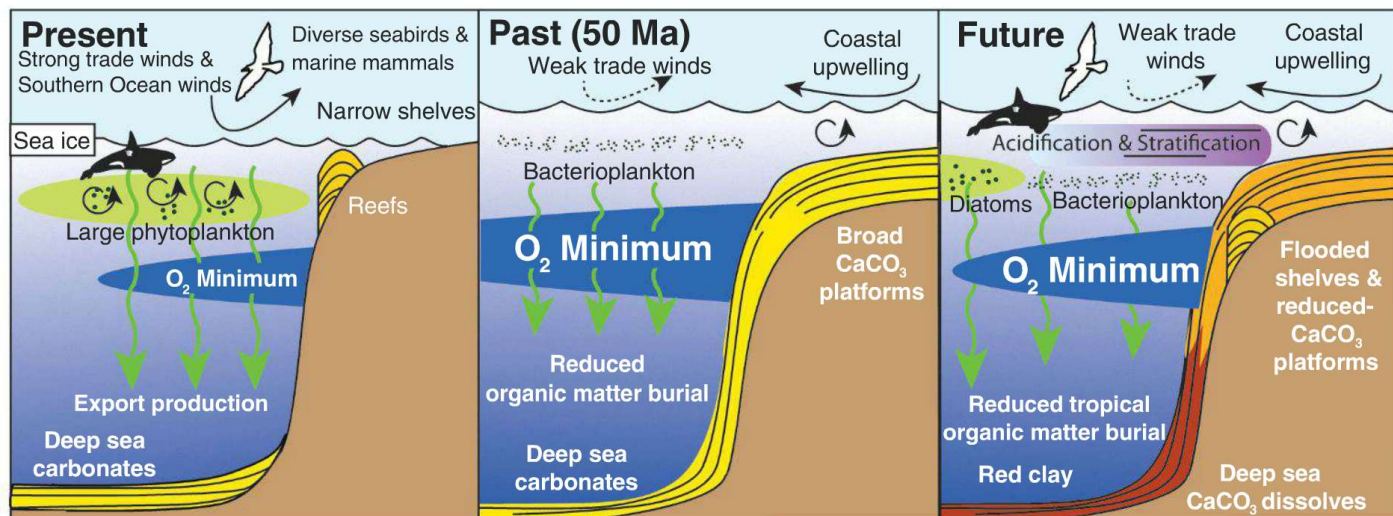


Fig. 1. Comparison of present, past, and future ocean ecosystem states. In the geologic past (middle panel), a warmer, less oxygenated ocean supported longer food chains based in phytoplankton smaller than present-day phytoplankton (left panel). The relatively low energy transfer between trophic levels in the past made it hard to support diverse and abundant top predators dominated by marine mammals and seabirds, and also reduced deep-sea organic matter burial. Equilibration of weathering with high atmospheric $p\text{CO}_2$ allowed carbonates to accumulate in parts of the deep sea. Reef construction was limited by high temperatures and coastal runoff even as high

sea level created wide, shallow coastal oceans. In the future (right panel), warming will eventually reproduce many features of the past warm world but will also add transient impacts such as acidification and stratification of the surface ocean. Acidification will eventually be buffered by dissolving carbonates in the deep ocean, which create carbonate-poor “red clay.” Stratification and the disappearance of multiyear sea ice will gradually eliminate parts of the polar ecosystems that have evolved in the past 34 million years and will restrict the abundance of short-food chain food webs that support marine vertebrates in the polar seas.

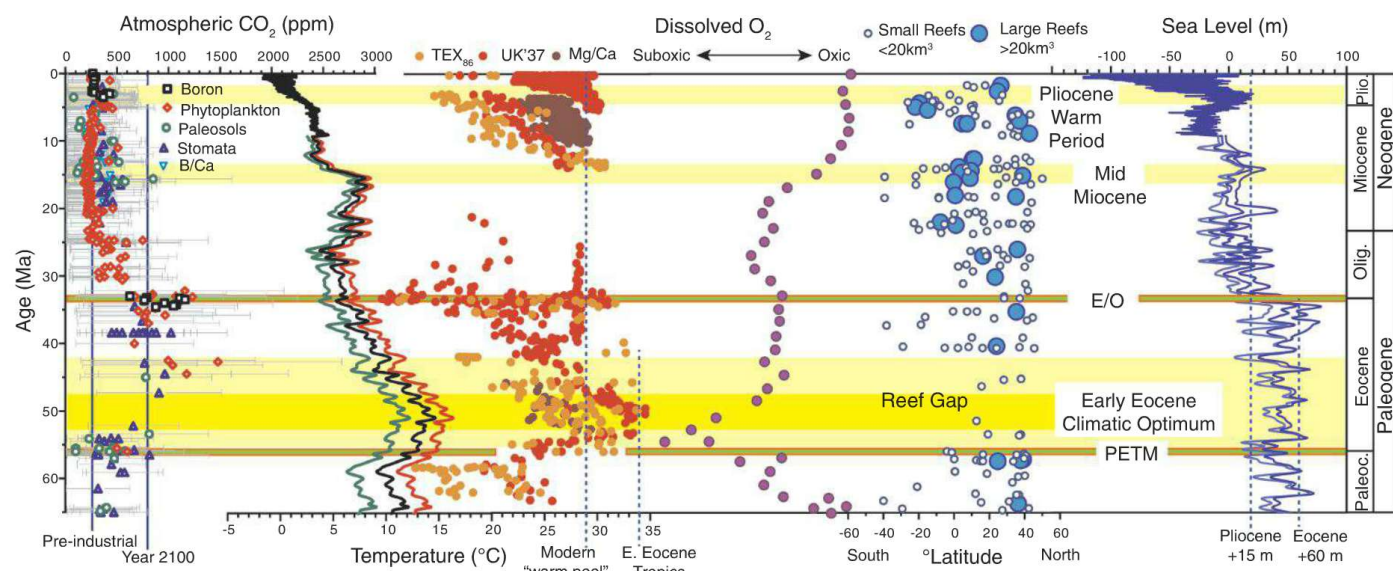


Fig. 2. Cenozoic record of major ecosystem drivers and responses. From left to right: Proxy evidence for atmospheric $p\text{CO}_2$ (14); vertical lines show the preindustrial $p\text{CO}_2$ (280 ppm) and estimated $p\text{CO}_2$ for year 2100 under a business-as-usual emissions scenario. Deep ocean temperature (15) estimated from oxygen isotope and Mg/Ca proxy evidence. Black line is long-term average for benthic foraminifera, red line is temperature adjusted for pH, and green line is temperature adjusted for seawater $\delta^{18}\text{O}$ effects. Compilation of surface ocean temperature estimates from multiple organic matter and Mg/Ca proxies (as indicated by different colors) (37, 90–98); data are plotted with their published

assumptions and age models. (Although the age models may differ slightly, these differences are not apparent at this resolution.) Dissolved O_2 is estimated from the Benthic Foraminifer Oxygen Index in a global compilation (99). Reef volume and temporal distribution (31); reefs with estimated volumes greater than 20 km^3 are plotted in large solid circles, smaller reefs as open circles. Sea level (15) is estimated from combined benthic foraminifer $\delta^{18}\text{O}$, Mg/Ca, and the New Jersey sea-level record: long-term average (middle line) and uncertainty (maximum, minimum lines). Dotted vertical lines show the estimated average high stands for the Pliocene and Eocene.

pelagic predators (including the whales, seals, penguins, and tunas) began in the greenhouse world of the late Cretaceous and early Paleogene, their modern forms and greatest diversity were achieved later, as large diatoms emerged as important primary producers and food chains became shorter and more diverse in the icehouse world (Fig. 3).

Multiple lines of evidence suggest that early Paleogene (45 to 65 Ma) ecosystems differed from the modern with respect to the organic carbon cycle (23, 24). The greenhouse ocean was about as productive as today, but more efficient recycling of organic carbon led to low organic carbon burial (23, 25). There were also major radiations of midwater fish, such as lanternfish and anglerfish (26), and diversifications of planktonic foraminifer communities typical of low-oxygen environments between 45 and 55 Ma (27, 28) (Figs. 2 and 3).

Coral-algal reef systems existed throughout the low- to mid-latitude Tethys Seaway from ~58 to 66 Ma (Fig. 2) (29). During the very warm, high- $p\text{CO}_2$ interval between ~42 and 57 Ma, coral-dominated large reef tracts were replaced by foraminiferal-algal banks and shoals (30, 31). This “reef gap” was present throughout Tethys, Southeast Asia, Pacific atolls, and the Caribbean (29, 31–33). The timing of the Tethyan reef gap suggests that the loss of architectural reefs was related to some combination of unusual warmth of the tropics and hydrologic changes in sedimentation and freshwater input (34, 35). Notably,

many modern groups of reef fishes evolved before or during this time (26, 36), which suggests that the changing biogeography of metazoan reefs and extensively flooded continental shelves may have contributed to their evolution.

Icehouse World Physical Conditions

Atmospheric $p\text{CO}_2$, which had been ~700 to 1200 ppmv during the late Eocene, fell to 400 to 600 ppmv across the Eocene-Oligocene boundary (34 Ma) (37). The decline in greenhouse gas forcing caused tropical SSTs to fall to values within a few degrees of those in the modern “warm pool” western Pacific or western Atlantic (~29° to 31°C) by 45 Ma (37) (Fig. 2). At high latitudes, deep ocean temperatures declined to 4° to 7°C between 15 and 34 Ma, with further polar cooling over the past 5 million years (15).

Polar cooling and Antarctic ice growth between ~30 and 34 Ma occurred as CO_2 levels declined (38) and were accompanied by the tectonic separation of Antarctica from Australia and eventually South America (17). Establishment of the Antarctic Circumpolar Current increased the pole-to-equator temperature gradient, increased the upwelling of nutrients and biogenic silica production in the Southern Ocean, and initiated modern polar ecosystems (39, 40). The growth of polar ice at ~34 Ma produced a sea-level fall of ~50 m, and the later growth of Northern Hemisphere ice sheets at 2.5 Ma initiated a cycle of sea-level fluctuations of up to 120 m (41, 42). In the Arctic, the ecosystem evolved from a marine anoxic “lake”

to a basin with perennial sea ice cover by at least 14 Ma (43).

Icehouse World Ecosystems

By 34 Ma, an ecosystem shift occurred in the high southern latitudes (40) as better wind-driven mixing in the Southern Ocean supported diatom-dominated food chains. The resulting short food chains fueled a major diversification of modern whales (39, 44), seals (45), seabirds (46, 47), and pelagic fish (48) (Fig. 3). The onset of Southern Ocean cooling is closely timed with the appearance of fish- and squid-eating “toothed” mysticetes at ~23 to 28 Ma and the radiation of large bulk-feeding baleen whales beginning at ~28 Ma (49). It is hypothesized that tropical and upwelling diatom productivity—initiated by nutrient leakage out of the high latitudes—spurred the development of long-distance migration by the great whales in the past 5 to 10 million years (39). This interval also coincides with radiations of delphinids (50), penguins (46), and pelagic tunas (48) (Fig. 3). The diversification of Arctic and Antarctic seals also unfolded during the past 15 million years as polar climates intensified and sea ice habitats expanded (45).

Reefs expanded in low to mid-latitudes, particularly in the northern subtropical Mediterranean and western Pacific, by ~42 Ma (31). However, the major growth of large reefs mostly occurred from ~20 Ma to the present, with major expansions of reef tracts in the southwestern

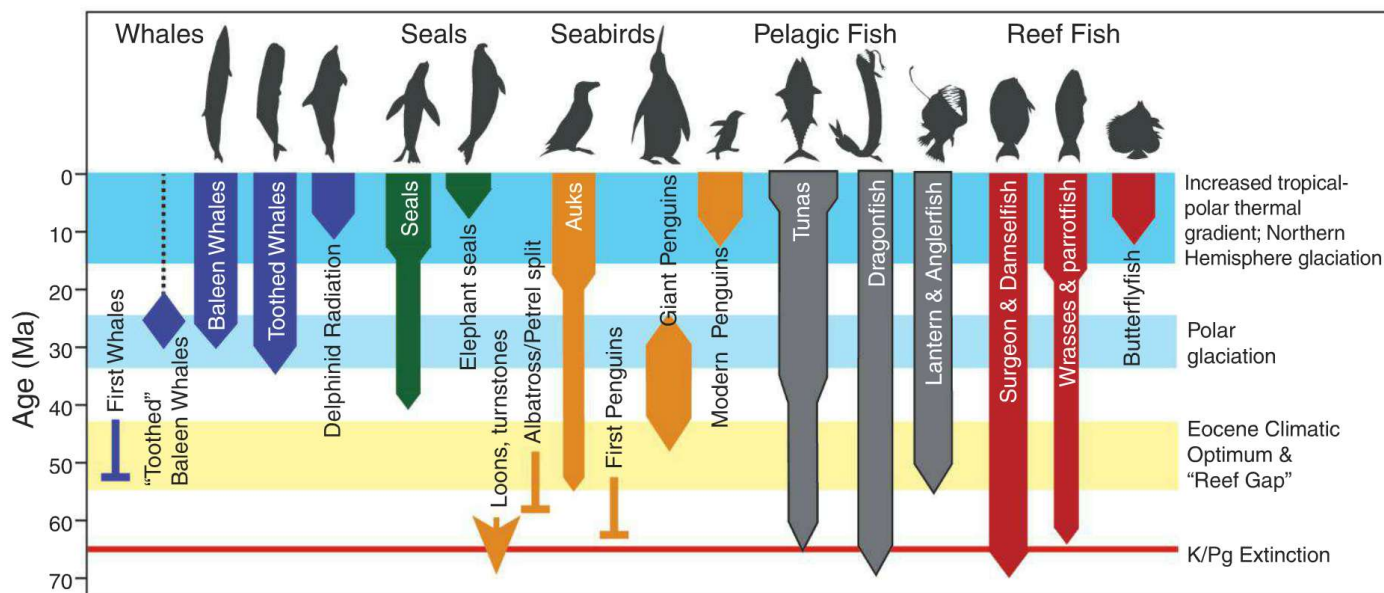


Fig. 3. Evolutionary events and diversification of selected marine vertebrate groups. Radiations of marine birds, tuna, mid-pelagic fish (e.g., dragonfish), and various groups of reef fish occur at or before the Cretaceous-Paleogene (K/Pg) mass extinction (65 Ma). The Eocene Climatic Optimum (45 to 55 Ma) is associated with the first whales (44), radiations of pelagic birds [albatrosses (100), auks (47), and penguins (46)], and diversification of midwater lanternfish and anglerfish (26).

With the onset of Antarctic glaciation (~34 Ma) and development of the Circum-Antarctic current, there is the major diversification of whales (49) and giant, fish- and squid-eating penguins (46). The differentiation of polar and tropical climate zones at ~8 to 15 Ma is associated with the extensive diversification of coastal and pelagic delphinids (50), Arctic and Antarctic seals (45), auks (47), modern penguins (46), pelagic tuna (48), and reef fishes (52, 53).

Pacific and the Mediterranean (31, 51). Several large groups of reef-associated fish, such as wrasses (52), butterflyfish (53), and damselfish (51), experienced radiations between 15 and

20 Ma (Fig. 3). These fishes include reef obligates, such as clades associated with coral feeding, that diversified along with the geographic expansion of fast-growing branching corals (53).

Sea-level variations in the past 34 million years had major impacts on shaping shallow marine ecosystems. The break in slope between the gentle shelf and the relatively steep slope

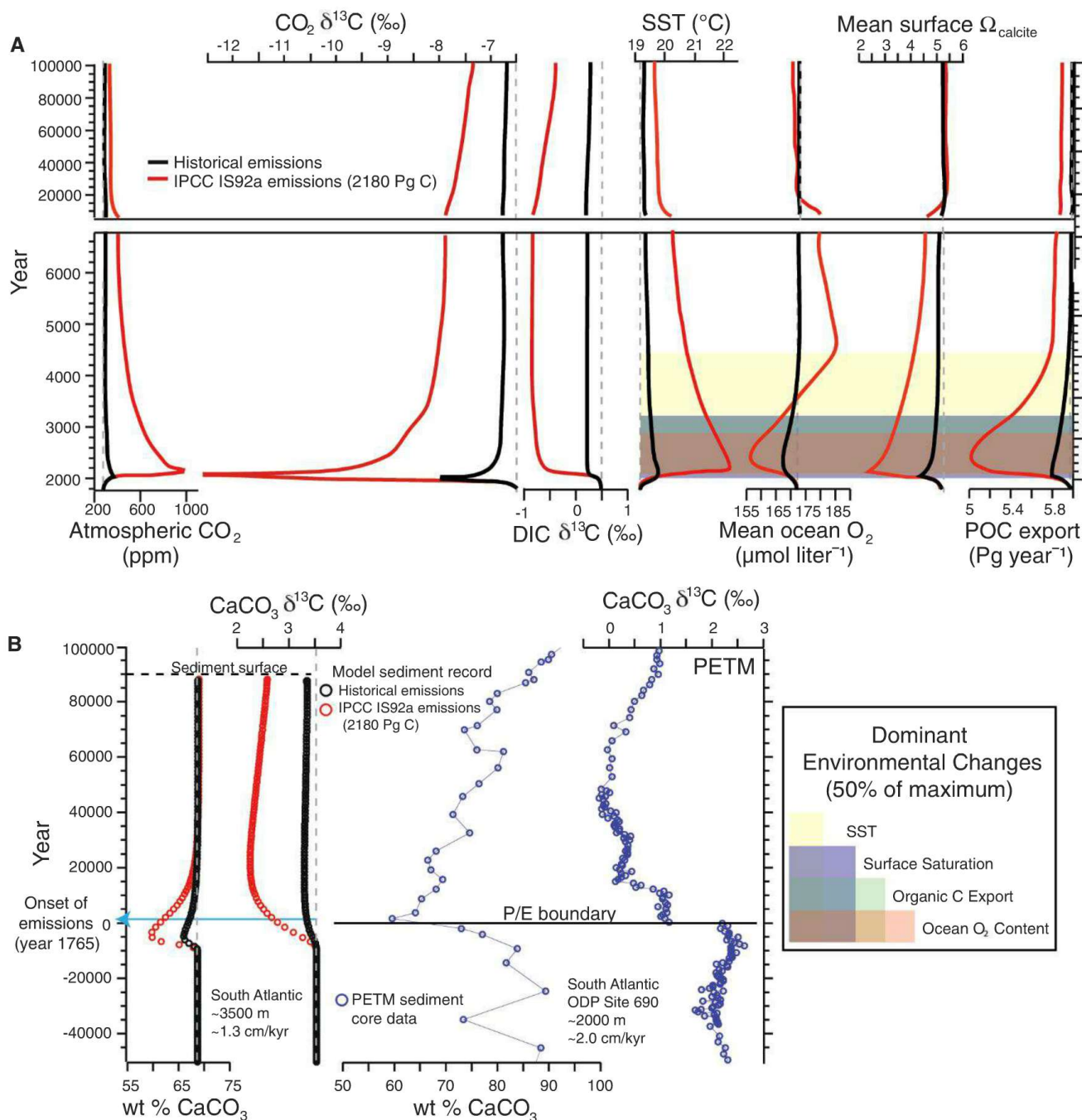


Fig. 4. Future Earth model. (A) 100,000-year simulations of historical anthropogenic CO₂ emissions (black lines) and the IPCC IS92a emissions (red lines). From left to right: the evolution of atmospheric CO₂, the δ¹³C of CO₂, the δ¹³C of dissolved inorganic carbon (DIC), SST, mean ocean O₂, surface ocean carbonate saturation with respect to calcite, and particulate organic matter (POC) export (modeled from a simple relationship with phosphate inventories). Note that some records such as the δ¹³C of DIC do not recover fully in even 100,000 years. Color bars highlight the interval in which each environmental variable (SST, mean ocean O₂, calcite saturation state, and organic C export) shows at least 50% of

the total change. (B) Modeled sedimentary expressions of the scenarios shown in (A) from a model site with a sedimentation rate of 1.3 cm per 1000 years (typical of the deep sea). Profiles show the weight percent CaCO₃ in sediments (left) and the δ¹³C of sedimentary carbonates (right). Although both scenarios were initiated in year 1765 (to simulate the start of the Industrial Revolution), dissolution of sea-floor carbonates and mixing by burrowers shifts the apparent start date substantially earlier than this. The PETM (blue lines, right) has a CaCO₃ minimum (101) and a δ¹³C minimum (102) comparable in duration, but larger in magnitude, than the simulated future.

occurs at ~100 m on most continental margins. In the later Pleistocene, glacially driven sea-level falls produced major decreases in shallow marine habitat area, fragmented formerly contiguous ranges of coastal species, and disrupted barrier reef growth (54–56). Sea level rose during glacial terminations at a pace of ~1 m per 100 years (57), a pace readily matched by the dispersal of benthic marine invertebrates and algae. However, Pleistocene sea-level rise was fast enough to trap sand in newly formed estuaries and create transient ecosystems such as marshes, sandy beaches, and sand-covered shelves over time scales of several thousand years (11, 55).

Lessons from Cenozoic Mean States

Past warm climates had warmer-than-modern tropics, extensively flooded continental margins, few architectural reefs, more expansive midwater suboxic zones, and more complete recycling of organic matter than now. Tropical oceans were likely as productive as today, but the productive waters expanded into the subtropics and supported long food chains based in small phytoplankton. Today, the inefficiency of energy transfer in picophytoplankton-dominated ecosystems limits the overall size of top predator populations and probably did so in the past. Although some of these conditions may recur in the future, tectonic boundary conditions are likely to prevent polar ecosystems from completely reverting to their past greenhouse world configurations in the near future. Hence, it seems unlikely that the Arctic “lake” will be reestablished or that wind-driven mixing will diminish enough in the Southern Ocean to destroy ecosystems founded on short, diatom-based food chains.

Past Transient Global Change Impacts: The Paleocene-Eocene Thermal Maximum

One of the best-known examples of a warm climate transient is the Paleocene-Eocene Thermal Maximum (PETM), a period of intense greenhouse gas-fueled global change 56 million years ago. The PETM is characterized by a 4° to 8°C increase in SSTs, ecosystem changes, and hydrologic changes that played out over ~200,000 years (58). Warm-loving plankton migrated poleward, and tropical to subtropical communities were replaced by distinctive “excursion” faunas and floras (35). Open-ocean plankton were dominated by species associated with low-productivity settings, whereas shallow shelf communities commonly became enriched in taxa indicative of productive coastal environments (59). Numerous in-

dicators suggest that toward the close of the PETM (100,000 years after it began), there was a widespread increase in ocean productivity (60, 61) and a transient rise in the carbonate saturation state above pre-PETM levels (62) due to intensified chemical weathering during the event (63).

The only major extinctions occurred among deep-sea benthic foraminifera (50% extinction) (35, 64). Surviving benthic foraminifera reduced their growth rate, increased calcification, and switched community dominance toward species accustomed to high food supplies and/or low-oxygen habitats (65). Deep-sea ostracodes also became dwarfed and shorter-lived, and many species vacated the deep sea into refugia for the duration of the PETM (35, 66). The preferential extinction of benthic foraminifera and temporary disappearance of ostracodes in the deep sea is attributed to a combination of a drop in export production associated with stratification in low and mid-latitudes, a marked drop in deep-sea dissolved oxygen concentrations related to transient ocean warming, and/or reduced carbonate saturation related to uptake of atmospheric CO₂ inventories (35, 64, 66, 67).

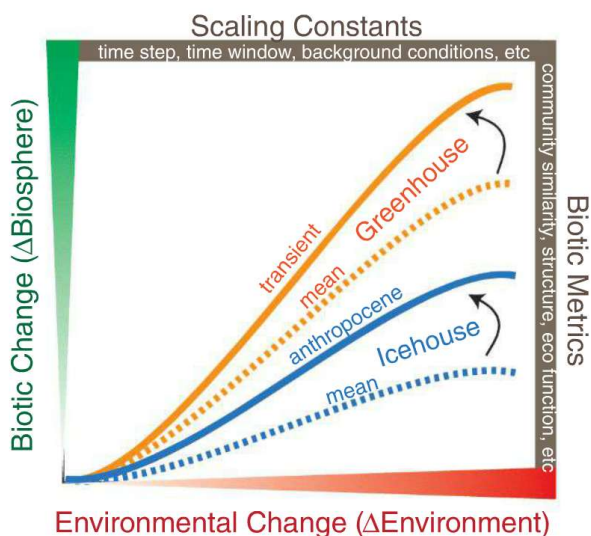


Fig. 5. Hypothetical biotic response to global change. Biotic sensitivity describes the response of ecosystems to a given amount of environmental change. It is currently unknown whether biotic sensitivity is constant in differing background conditions. For instance, here we illustrate the possibility that biotic sensitivity changes with climate state. This could occur if species exhibited broader environmental tolerances in icehouse climates, conferring greater biotic resilience across communities more generally. Modern global change is expected to change ecosystem structure in the direction of past greenhouse transient events. Additional research is needed to determine whether biotic sensitivity varies by taxa and ecosystem metrics (biotic metrics), sampling intervals, study durations, and background conditions (scaling constants). Our understanding of biotic sensitivity will determine our ability to predict future biotic changes over the next 50,000 to 100,000 years.

There are indications of a possible drop in carbonate saturation during the PETM, such as the common occurrence in a few species of mal-formed calcareous phytoplankton liths and planktic foraminifera (68). However, the evolution of reef ecosystems through the PETM argues against severe surface-ocean acidification. For instance, a Pacific atoll record does not record a distinct sedimentary change or dissolution event (69). In the Tethys Seaway, large coral-algal reefs disappeared prior to the PETM, but coral knobs persisted into the early Eocene amid the dominant foraminiferal-algal banks and mounds (34, 35). Hence, if there was surface-ocean acidification during the PETM, its effects were modest and did not precipitate a major wave of extinction in the upper ocean.

The Geologic Record of the Future: Diagnosing Our Own Transient

How representative are transient events like the PETM for Earth’s near future? We compared the PETM record to a modeled future of the historical record of CO₂ emissions (Fig. 4A, black lines) and the Intergovernmental Panel on Climate Change (IPCC) IS92a emissions scenario (peak emissions rate of 28.9 Pg C year⁻¹ in 2100; total release of 2180 Pg C) (Fig. 4A, red lines). The latter represents a conservative future, given the availability of nearly twice as much carbon in fossil fuels.

We used the Earth system model cGENIE, including representation of three-dimensional ocean circulation, simplified climate and sea ice feedbacks, and marine carbon cycling (including deep-sea sediments and weathering feedbacks) (70, 71). In both long-term (10,000 years) (10) and historical perturbation (72) experiments, cGENIE responds to CO₂ emissions in a manner consistent with higher-resolution ocean models. Experiments were run after a 75,000-year model spin-up, needed to fully equilibrate deep-sea surface sediment composition.

Under the IPCC IS92a emissions scenario, CO₂ peaks at ~1000 ppmv just after year 2100 (Fig. 4A). The emission of a large mass of fossil fuel carbon causes a ~6‰ drop in atmospheric δ¹³C and a ~1‰ drop in carbonate δ¹³C—an event less than half the size of the PETM anomaly (73). SSTs increase by 3°C, reaching a maximum just before year 2200, and remain elevated above preindustrial SSTs by almost 0.5°C for >100,000 years (Fig. 4A). Here again, the estimated PETM surface ocean temperature anomaly is larger, at 5° to 9°C (58, 73). In the future scenario, the CO₂ invasion of the surface ocean causes the mean calcite saturation state of the surface ocean (Ω) to drop to a minimum of 2 Ω just after year 2100,

accompanied by a decrease in CaCO_3 export. The global average hides the extensive regions of the world ocean that will become undersaturated with respect to calcite and, in particular, aragonite (74)—with profound biological effects (75, 76), including the possible cessation of all coral reef growth (77). Eventually, a temporary buildup of weathering products leads to an overshoot in Ω beginning around year 13,500 that persists through the remaining 100,000 years—an effect also observed after the PETM (78, 79). Mean ocean oxygen concentration is modeled to drop to a minimum at year 2500, return to preindustrial levels a thousand years later, and then increase above modern values for ~12,000 years as a result of the resump-

tion of strong ocean overturning and decreased export of particulate organic carbon. Both a drop in O_2 and export production also occurred in the early stages of the PETM (35).

The modeled sedimentary expression of the future is broadly similar to the PETM (Fig. 4B). The decrease in carbonate $\delta^{13}\text{C}$ and weight percent CaCO_3 is greater for the PETM than for the modeled future, consistent with a greater mass of carbon injected during the PETM than in the conservative future emissions scenario of 2180 Pg C. A striking aspect of the modeled future record is that the onset of the $\delta^{13}\text{C}$ excursion does not appear notably more rapid than for the PETM. Both sediment mixing (bioturbation) and carbonate dissolution act to shift the apparent onset of a

transient event into geologically older material and reduce the apparent magnitude of peak excursions (80, 81).

Biotic Sensitivity and Ecosystem Feedbacks

Ecological and environmental records from past oceans provide guideposts constraining the likely direction of future environmental and ecological change. They do not, however, inform key issues such as the likelihood that ecosystems will change or how they may mitigate or amplify global change. These are all questions of biotic sensitivity and ecosystem feedbacks.

Biotic sensitivity describes the equilibrium response of the biosphere to a change in the environment (Fig. 5). There is tantalizing evidence that ecosystem responses scale with the size of transient warming events in the same way that surface temperature scales with $p\text{CO}_2$ concentrations [i.e., climate sensitivity (82)]. Specifically, Gibbs *et al.* (83) found that change in nanoplankton community structure (Σ_{CV}) scaled with the magnitude of environmental change (as measured by $\delta^{13}\text{C}$) during a succession of short-lived global change events between 53.5 and 56 Ma. This result suggests that “background” biotic sensitivity can predict responses to much larger perturbations. Additional studies of biotic sensitivity in deep time are urgently needed to test whether this type of scaling exists across taxa and different ecosystems or with changes in background conditions, time scale, or time step (Fig. 5).

Ecosystem feedbacks have the potential to mitigate or amplify the environmental and ecological effects of current greenhouse gas emissions. For instance, the greenhouse gas anomaly of the PETM is drawn down more quickly than would be expected by physical Earth system feedbacks alone (60, 84). Widespread evidence for a burst in biological productivity in the open marine environments (60, 61) and indirect evidence for increased terrestrial carbon stores during termination of the PETM (84) support the hypothesized importance of negative ecosystem feedbacks in driving rapid carbon sequestration. Species-specific responses to environmental perturbation—including growth rates (85), dwarfing (86), range shifts (87), or loss of photosymbionts (88)—can affect the structure and function of entire ecosystems. For instance, ecological interactions are hypothesized to have an important influence in setting the carbonate buffering capacity of the world’s ocean (70, 71) and in driving Cenozoic-long trends in the carbonate compensation depth (89), among many others. The geological record of transient events has the largely unexploited potential to constrain the type and importance of ecosystem feedbacks.

Lessons for the Future

The near future is projected to be a cross between the present climate system and the Eocene-like

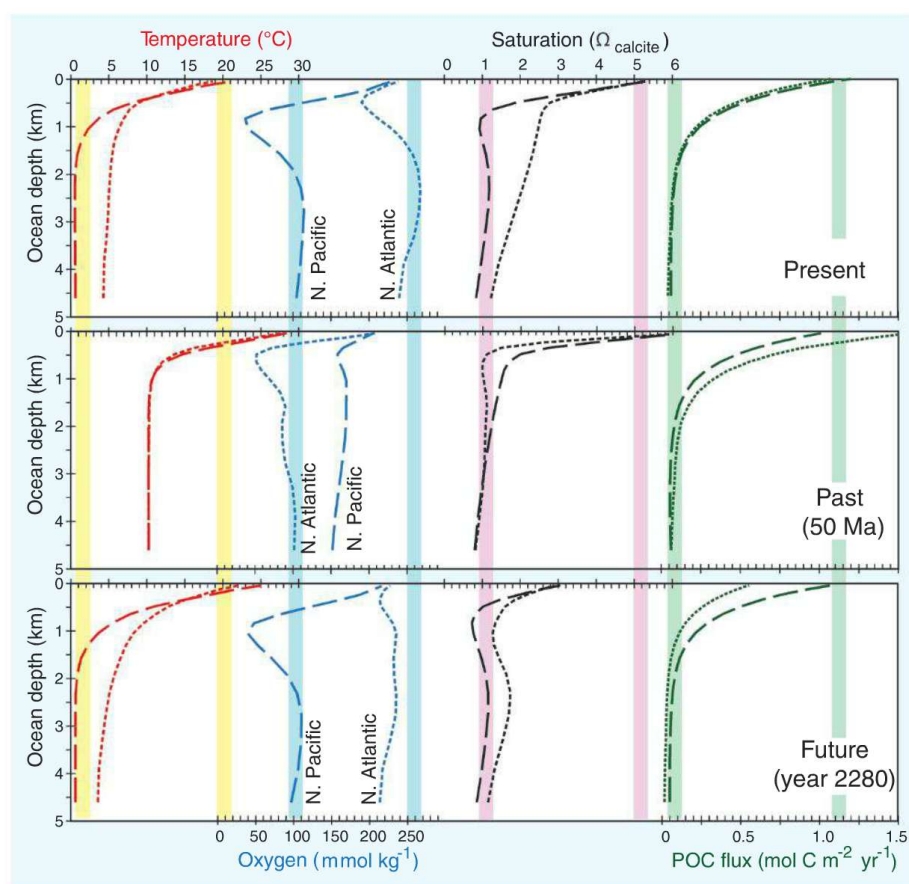


Fig. 6. Dynamics of past, present, and future oceans. Profiles of ocean temperature (red lines), oxygen saturation (blue lines), carbonate ion saturation (black lines), and particulate organic carbon flux (green lines) are shown for modeled averages of the North Pacific (long dashes) and the North Atlantic (short dashes). Note that the future (bottom panel) is a hybrid of the present (top panel) and the past (middle panel). Although the future SST resembles that at 50 Ma, the future deep ocean is much colder, reflecting the continued presence of polar ice and an Antarctic Circumpolar Current. Future oxygenation is reduced in the Atlantic relative to the present, but not as much as in the 50 Ma simulation. Notably, surface ocean carbonate saturation is much lower in the future relative to the present or past, reflecting ocean acidification. Eventually, acidified surface waters will be neutralized by reacting with deep-sea carbonates, reducing deep-sea carbonate concentrations by 10 to 20% (see Fig. 4B). POC flux is model-dependent but shows the expected drop in organic matter export in the future ocean. Colored vertical bands show the maximum and deep ocean minimum values for each parameter. Profiles were generated in cGENIE, with the future simulation the same as in Fig. 4A.



warmth of coming centuries (Fig. 6). Our future Earth model and analogies to the PETM show that a transitional, non-analog set of climates and ecosystems will persist for >10,000 years because of the slow response times of many parts of the biosphere. Over this interval, the oceans will continue to take up CO₂ (from fossil fuel combustion) and heat, causing a rise in sea level, acidification, hypoxia, and stratification. Lessons from the PETM raise the possibility that extinctions in the surface oceans due to greenhouse gas-driven Earth system change will be modest, whereas reef ecosystems and the deep sea are likely to see severe impacts (2). In addition, Earth now supports a much more diverse group of top pelagic predators vulnerable to changes in food chain length (9) than it did in the PETM. The severity and duration of ecosystem impacts due to human greenhouse gas emissions are highly dependent on the magnitude of the total CO₂ addition. If the CO₂ release is limited to historical emissions, ocean surface temperature and carbonate saturation will return close to background within a few thousand years, whereas the “conservative” modeled 2180 Pg C release produces impacts persisting at least 100,000 years (Fig. 4B). Although the future world will not relive the Eocene greenhouse climate, marine ecosystems are poised to experience a nearly continuous state of change lasting longer than modern human settled societies have been on Earth.

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REVIEW

Climate Change and the Past, Present, and Future of Biotic Interactions

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Biotic interactions drive key ecological and evolutionary processes and mediate ecosystem responses to climate change. The direction, frequency, and intensity of biotic interactions can in turn be altered by climate change. Understanding the complex interplay between climate and biotic interactions is thus essential for fully anticipating how ecosystems will respond to the fast rates of current warming, which are unprecedented since the end of the last glacial period. We highlight episodes of climate change that have disrupted ecosystems and trophic interactions over time scales ranging from years to millennia by changing species' relative abundances and geographic ranges, causing extinctions, and creating transient and novel communities dominated by generalist species and interactions. These patterns emerge repeatedly across disparate temporal and spatial scales, suggesting the possibility of similar underlying processes. Based on these findings, we identify knowledge gaps and fruitful areas for research that will further our understanding of the effects of climate change on ecosystems.

Climate change has occurred repeatedly throughout Earth's history, but the recent rate of warming far exceeds that of any previous warming episode in the past 10,000 years (1, 2) and perhaps far longer. Knowledge of how climate change has altered interactions among organisms in the past may help us understand whether consistent patterns emerge that could inform the future of a warming and increasingly human-dominated planet. The fossil record provides an opportunity to study ecosystems on both ecological and geological time scales but is unevenly distributed across time, environments, and taxa and contains only fragmentary information about biotic interactions (3). Modern systems provide direct, though short-term, observational (4) and experimental (5, 6) evidence of changes in biotic interactions during climate change that together can elucidate important mechanisms driving ecological and evolutionary processes. However, it is not always clear how to extrapolate the insights gained from short-term observations over the longer time scales on which future climate change will play out. Robust predictions about the future require multispecies models that combine long-term insights from the past with more specific and shorter-term insights from modern systems—a herculean challenge, given that models for species responses to climate change have only begun to incorporate biotic interactions (7). Even the term “biotic interactions” means different things to different disciplines. We view biotic interactions

in broad terms—namely, as the influence of individuals or populations on one another. In practice, observations from the fossil record and models of the future generally consider the potential interactions of co-occurring species, whereas actual interactions are more easily identified in modern systems. Here we combine insights from past and present-day ecological systems to understand how climate change has affected biotic interactions through time and to identify fruitful avenues for adequately predicting future changes to ecosystems.

How Did Past Climate Change Alter Biotic Interactions?

The geologic record provides unambiguous evidence that some past episodes of climate change have altered biotic interactions by driving extinction and speciation and altering the distributions and abundances of species. The relative diversities of clades and functional groups have varied enormously over geological time [for example, see Fig. 1 for marine genera (8)], and these diversity changes were often accompanied by changes in biotic interactions at both local (9) and global (8, 10) scales. Marine ecosystems, which have the most complete fossil record, exhibit long intervals of relative stability in broad ecological and taxonomic structure, punctuated by short episodes of turnover and ecological upheaval (Fig. 1). These episodes are the well-known mass extinction events (Fig. 1) (11), several of which appear to have resulted from climate change and associated changes such as ocean acidification, eutrophication, and anoxia (12–15).

Mass extinctions illustrate the outcome of complex nonlinear feedbacks between climate change and biotic interactions and offer insights into the types of biotic changes that may be expected in the future. One recurring motif in both marine and terrestrial systems is community homogenization: Mass extinction events are often

followed by the establishment, sometimes for hundreds of thousands of years or longer, of assemblages dominated by ecological generalists with broad environmental ranges. The catastrophic Permian-Triassic (PT) extinction (Fig. 1) demonstrates this phenomenon: Rapid warming and ocean acidification probably caused the extinction of a large proportion of marine (12) and terrestrial (16) taxa, and in both realms post-extinction communities were dominated by ecological generalists (17, 18). Similarly, specialized plant-insect associations recovered much more slowly after the end-Cretaceous mass extinction (Fig. 1) and associated climatic changes (19) than did generalist associations (20).

Mass extinction events may continue to affect the structure of biotic interactions long after ecosystems have recovered to pre-extinction diversity levels. In the case of the PT extinction, the ecosystems that arose after the Early Triassic recovery interval show evidence of increased complexity relative to their pre-extinction analogs (16, 21). For example, in the terrestrial realm some vertebrate groups maintained their pre-extinction functional roles, but entirely new functional groups also emerged, in time giving rise to more complex networks of interactions than existed before the extinction (16). In the marine realm, the PT event profoundly altered the long-term diversity trajectories of major taxa (Fig. 1), and relative abundance distributions imply a lasting post-Permian increase in the ecological complexity of benthic communities (21).

Although mass extinctions provide some of the best evidence for altered biotic interactions, networks of biotic interactions (as implied by the composition of fossil assemblages) also change in ways that do not necessarily involve extinction. Climate-mediated dispersal and invasion events are prominent in the fossil record (22, 23) and may provide valuable analogs for the present. A particularly pertinent example is the Paleocene-Eocene Thermal Maximum 55 million years ago (Ma), when a sudden rise in atmospheric greenhouse gases drove rapid global warming (24). In the Bighorn Basin of North America, this event was associated with compositional changes and novel but transitory species assemblages that emerged after range shifts and the immigration of new species (22). In this same region and time frame, rising temperatures led to increased intensity and frequency of insect herbivory on plants (Fig. 2) (25). The link between insect damage and temperature through time is consistent with modern meridional gradients in herbivore damage diversity (26), suggesting that increased insect herbivory may be a persistent effect of future climate warming (25). The Great American Biotic Interchange, facilitated by a combination of tectonic changes from 12 to 3 Ma that formed the isthmus connecting North and South America and climate-driven changes in habitat along the isthmus, offers another example of large-scale faunal interchange (27). During this event, plants

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probably dispersed between North and South America several million years before animals (28), and rates of evolutionary diversification differed within immigrant mammals in North versus South America (27). These differences in dispersal and diversification among taxa suggest that the arrival of new species into each continent greatly modified existing biotic interactions. Transient, novel assemblages were also a common aspect of latest Pleistocene ecosystems (Fig. 2) (9, 29). The formation of novel plant assemblages in eastern North America (29, 30) appears to have been driven by both taxon-specific range and abundance shifts in response to Pleistocene climate change and ecological release after anthropogenically driven megaherbivore extinction (Fig. 2) (9). The persistence of these communities for almost 2000 years (Fig. 2) suggests that novel

assemblages formed by contemporary and future climate changes may be transitory on geological time scales but long-lived on human time scales.

Whereas changes in the distribution and abundance of species suggest underlying changes in biotic interactions, food web reconstructions inferred from functional morphology (31) or stable isotopes (32, 33) offer more concrete evidence. So far, only a handful of studies have directly evaluated changes in food web structure associated with climate change episodes. One such study suggests that the extinction of some large vertebrate groups during the PT events may have altered the structure of terrestrial food webs in ways that made the generalist-dominated post-extinction recovery communities more prone to ecological collapse (34). Stable isotopic approaches are more feasible in younger assemblages with better preservation

and are a promising area for future research. For example, isotope-based food web models indicate that predator-prey interactions changed with deglacial climate change, with some predators switching prey during the Last Glacial Maximum 21,000 years before the present (yr B.P.) and overall increases in specialization by predators (35).

How Does Contemporary Climate Change Alter Biotic Interactions?

Recent observations and experiments show that climatic changes on the scale of years to decades can change the distributions and abundances of species and alter biotic interactions. As in the past, contemporary climate change may lead to novel, altered, or lost interactions through (local) extinctions, range shifts, and changes in relative abundance (36, 37). For example, with rising tem-

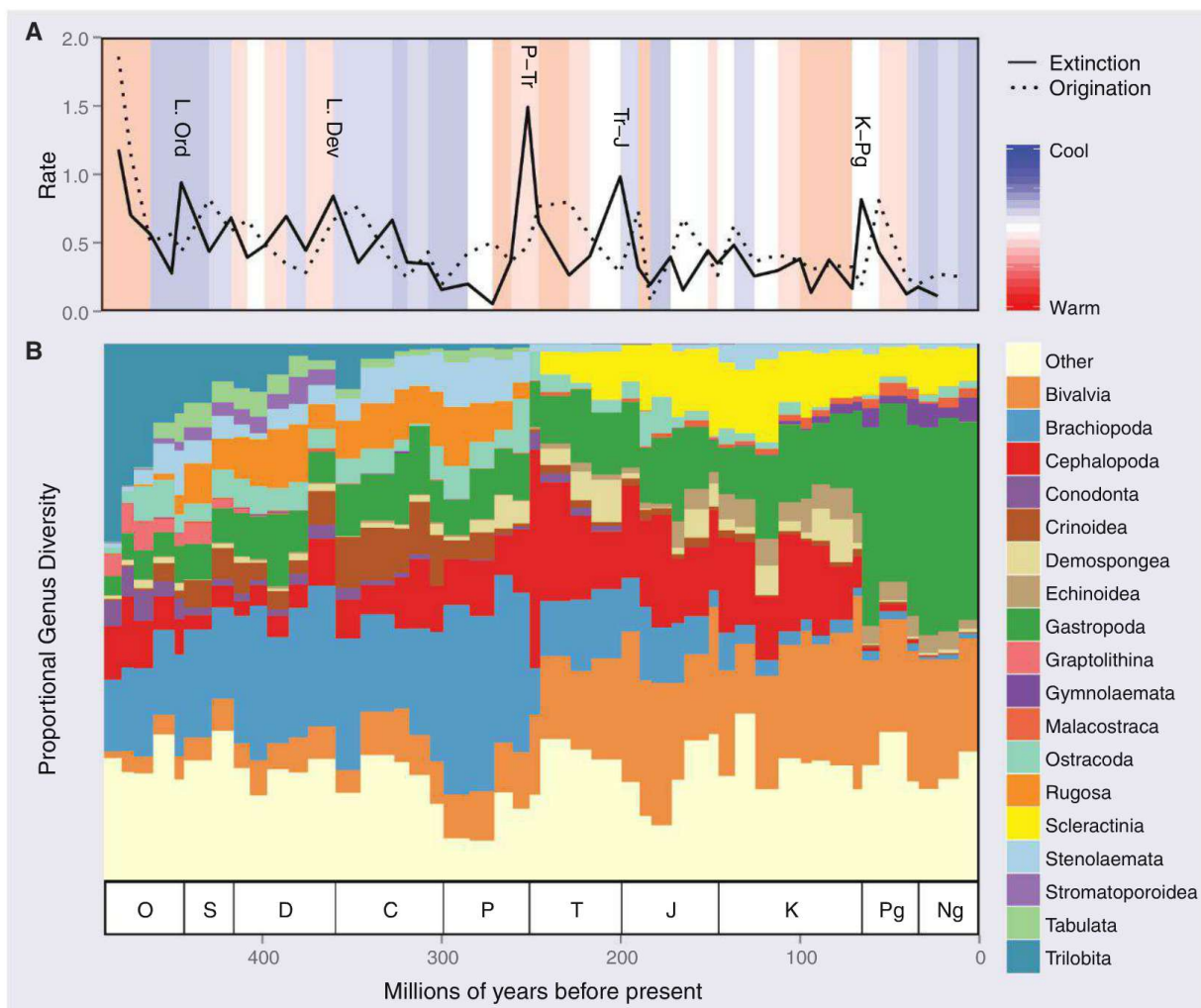


Fig. 1. Macroevolutionary rates and changes in the proportional diversity of fossil marine invertebrate taxa through time and their relationship to broad climate trends. (A) Rates of extinction (solid line) and origination (dotted line) from the Paleobiology Database (8, 85). Colored bands represent relatively warm (red) and cool (blue) intervals and are based on the mean oxygen isotope ratio ($\delta^{18}\text{O}$) of well-preserved marine skeletal carbonates (86) after detrending and rescaling to remove the poorly understood long-term Phanerozoic trend toward

heavier $\delta^{18}\text{O}$ values (86). The “Big Five” mass extinctions are indicated (L. Ord, Late Ordovician; L. Dev, Late Devonian; P-Tr, Permian-Triassic; Tr-J, Triassic-Jurassic; K-Pg, Cretaceous-Paleogene). **(B)** Proportional genus diversity through time, based on genera sampled within each time bin. Age in millions of years before the present and geological periods are indicated along the horizontal axis (O, Ordovician; S, Silurian; D, Devonian; C, Carboniferous; P, Permian; T, Triassic; J, Jurassic; K, Cretaceous; Pg, Paleogene; Ng, Neogene).

peratures, species co-occurrence can switch to competitive displacement (38), predation can intensify (39), or new predator-prey interactions can result (40). Fluctuations in climate can also dissipate biotic interactions and allow coexistence by favoring inferior competitors (36). In general, climate change should favor species able to tolerate warmer and more variable climatic conditions, resulting in a relative increase in their performance and/or movement to new locations.

Further complexities arise because feedbacks between biotic interactions and climate can lead

to larger changes in climate and ecosystem function. For example, changing levels of atmospheric CO₂ may alter the relative abundances of different vegetation functional groups such as woody versus nonwoody plants) and in turn affect ecosystem function even further (41). Warming experiments in the Arctic show that higher temperatures favor shrubs (42), and these changes in composition can alter regional climate through changes in albedo and evapotranspiration (43), a feedback that probably occurred during the mid-Holocene 6000 yr B.P. with expanding boreal forests (44).

By 2100, the areal extent of shrubs is expected to expand by 20% (45) to 52% (46) in areas north of 60° latitude, leading to regional temperature increases via decreased albedo and increased evapotranspiration (45, 46).

Higher trophic levels may be most sensitive to climatic change, and both modern and fossil evidence shows that disrupting their trophic interactions can amplify climate changes throughout the community (6, 9, 47). At the same time, experiments in aquatic systems show that warming can intensify trophic cascades, leading to stronger control by top consumers, especially keystone species (39, 48). For example, in pitcher plant communities, top-down controls were stronger with warmer temperatures (49) and in lower-latitude sites than in higher-latitude sites (50). However, climate only accounted for a small amount of the variability in food web structure within these communities along spatial environmental gradients (51). Overall, whether warming promotes or weakens trophic interactions, the results are likely to amplify throughout the community (47).

Climate-driven changes in phenology (the timing of life history events) are especially likely to alter trophic interactions (4), resulting in trophic mismatches (52) and community instability (6). For example, in parts of the Arctic, caribou mediate the effects of warming temperatures on plant functional groups by reducing shrubs and favoring forb production (6). Recent climate change has shifted the peak quality of tundra forage plants to earlier in the year, yet the timing of caribou calving in some regions has not kept pace (52), leading to trophic and phenological mismatches. Similar mismatches and/or new associations during climate change can also result from spatial mismatches due to differences in dispersal ability between interacting species (53). Vagile species are more likely to track changing climate, whereas dispersal-limited species generally are not (54), probably resulting in changes to biotic interactions (36, 53). The superior dispersal ability of a competitor can result in competitive release but also may lead to new competitive matches as novel communities form (37). In turn, these novel interactions could result in further changes to community composition because of a lack of coevolved history (36) (Fig. 3).

Can We Predict Future Biotic Interactions with Climate Change?

Given the interrelationships between climate change, biotic interactions, dispersal, and community composition, models of individualistic species-climate relationships alone will be insufficient to predict future ecological changes (53, 55). For example, adding occurrences of interacting species (prey availability and predator pressure) improved the performance of correlative species distribution models (SDMs) for the arctic fox (*Alopex lagopus*) in Scandinavia (56). Similarly, accounting for dispersal differences and adding a competitor to a SDM helped explain why arctic

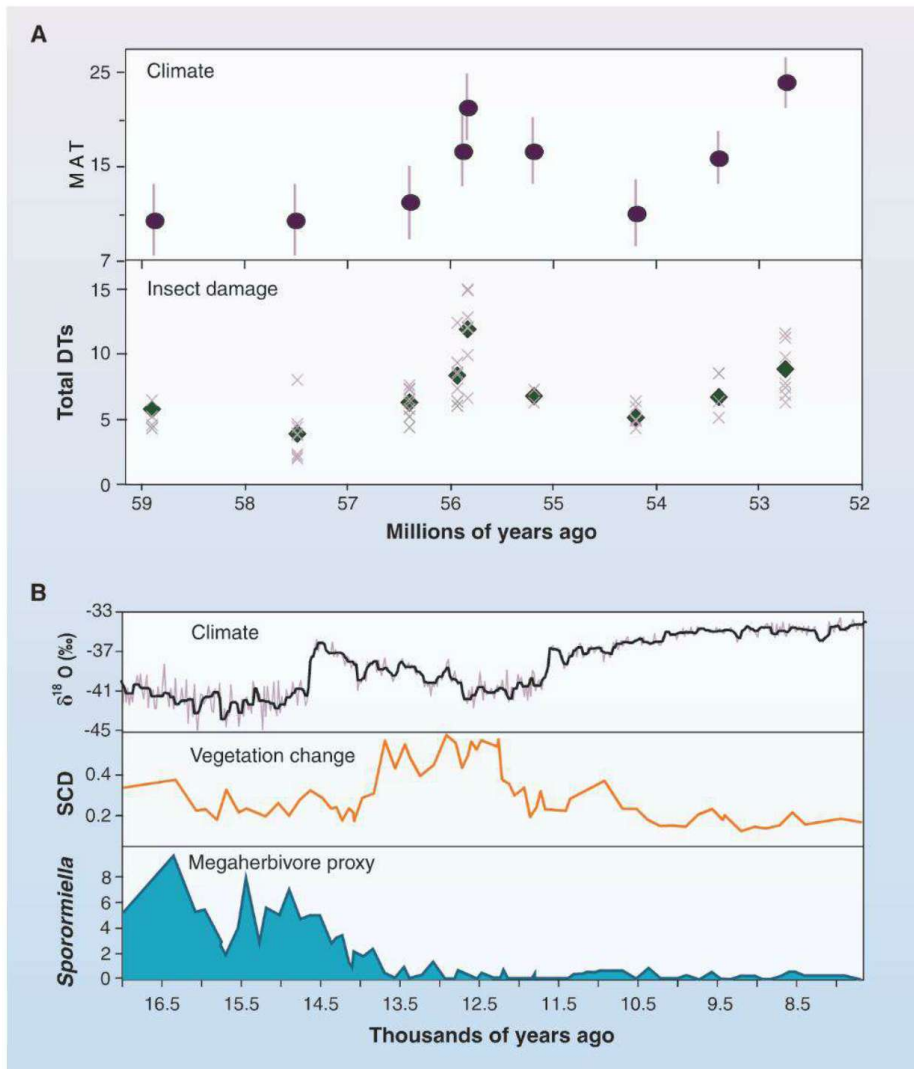


Fig. 2. Biotic interactions through time. (A) The top panel shows an index for mean annual temperature (MAT, ± 1 SD) based on leaf margin analysis, and the bottom panel shows the number of insect damage types (DTs) across the Paleocene-Eocene Thermal Maximum (PETM). Each X symbol represents the number of DTs on a plant host with at least 20 leaves in the flora; the diamonds are the means of the X's at the site [reprinted with permission from (25)]. Insect damage peaked with temperature rise at the PETM. (B) Megafaunal extinction and vegetation change across the Pleistocene-Holocene transition (9). The black line in the top panel indicates $\delta^{18}O$ from the North Greenland Ice Core Project (87). The orange line represents the minimum squared chord dissimilarity (SCD), indicating the dissimilarity of vegetation from that of the present. The blue line represents the abundance of the dung fungus *Sporormiella*, as a percentage of the upland pollen sum, which represents the presence or absence of megafauna. Vegetation dissimilarity peaked after local megafaunal extinction [reprinted with permission from (9)].

char (*Salvelinus alpinus*) may not expand into climatically suitable lakes as temperatures warm in the future (57).

Despite promising results from SDMs that include biotic as well as climatic predictors, there is a clear need to develop and validate more process-based methods that incorporate multi-species interactions, dispersal, and community assembly to predict communities of the future. Recent work suggests that this might best be realized by examining spatial and temporal patterns of species co-occurrence along environmental gradients (58) and by developing dynamic macroecological models that consider patterns of co-occurrence while incorporating (implicitly or explicitly) important ecological processes (59). Although a paucity of spatiotemporal co-occurrence data may challenge the parameterization and validation of such models (55), the relatively data-rich Quaternary (2.588 Ma to the present) represents an important exception. Pooling data across time may provide more robust estimates of species-climate relationships (60–62) and could distinguish species associations that arise because of similar environmental constraints from those due to tightly linked biotic interactions (63). Simplifying communities to assemblages of functional groups or traits may also help develop robust predictions that translate across time scales (64).

Opportunities for Synthesis

Whereas increased understanding of the ways in which climate change influences biotic interactions is key to making predictions about the future (36, 65), substantial challenges remain. A crucial difference between the past and the future is the degree of human alteration of ecosystems. Humans already influence more than 80% of Earth's land surface (66), and by 2100, when human population size is expected to double that of today, a quarter or more of the planet could experience climatic conditions that have no modern analog (67). The combination of climate change, human land use, and unsustainable harvests may ultimately lead to extinction rates rivaling those of major mass extinctions in the geological past (68). Mass extinctions have strongly affected the form and nature of ecosystems throughout time; given the interaction of diverse anthropogenic drivers today and in the future, and especially when considered alongside the ongoing global exchange and spread of invasive species, a future mass extinction event could be accompanied by community reorganization, homogenization, and ecological novelty on an unprecedented scale.

How, then, do we move forward toward a better understanding of the future of biotic interactions? Both the past and present provide important insights regarding the influence of climate change on biotic interactions. We highlight four areas of promising synthesis across time scales that can help anticipate changes in the future: (i)

compile baselines for the relative frequency of specialized versus generalized interactions through time; (ii) elucidate the role of dispersal in mediating changes in biotic interactions; (iii) focus on time-invariant metrics such as interactions between functional groups rather than species; and (iv) use the rich and high-resolution paleoclimatic and ecological data from the Quaternary as a bridge between the ecological time scales of the present and the evolutionary scales of deep time.

Across time scales, we lack baselines for the relative frequency of specialized versus generalized interactions and how that frequency will shift with climate change. For example, a long-held theory in ecology is that specialized interactions should be most prevalent in stable environments, where time and stability allow such tightly co-evolved interactions to arise and persist (69). In contrast, generalized interactions should dominate regions that have experienced rapid environmental change. Current global biogeographical patterns support these predictions (70), and regions where climate fluctuated more strongly during the Quaternary show community structures consistent with a history of disrupted spe-

cies interactions (71). Additionally, generalist taxa (72) and interactions (20) often dominated assemblages after rapid past climate change. When extrapolated to the rapidly changing conditions of the future, tightly coevolved interactions—notably mutualism and parasitism—could be under greatest threat (36, 73). Given the projected combination of highly novel environments (67) with increasing impacts from other anthropogenic drivers (74), rapid biotic turnover, especially where weedy species and pathogens are poised to invade disturbed or weakly coevolved systems, may result in the formation of communities and ecosystems very different from those on Earth today (Fig. 3) (75). The combined impacts of extinction and invasion also mean that communities will become increasingly homogeneous in the future (76), at least on short evolutionary time scales. However, key issues need to be resolved before we can fully generalize this prediction. First, the definition of what constitutes a “generalist” or “weedy” species or interaction needs to be reconciled across paleo and modern systems. Second, limited evidence from mass extinction events suggests that more-complex ecosystems emerge after the transient rise of generalist taxa,



Fig. 3. Climate change and biological invasions alter the distribution and abundance of species, resulting in novel species combinations and interactions between organisms with no previous history of association. (A) Recent increases in minimum winter temperature have allowed the palm *Trachycarpus fortunei* to escape cultivation and invade the deciduous forest of southern Switzerland, far north of other viable palm populations (88) [photo credit: M. C. Fitzpatrick]. Novel interactions between species can sometimes cause dramatic and unpredictable changes in ecosystems. By removing the dominant native omnivore, the red land crab (*Gecarcoidea natalis*), and by increasing the populations of two scale insects (*Tachardina aurantiaca* and the nonnative *Coccus celatus*), the invasion of the yellow crazy ant (*Anoplolepis gracilipes*) on Christmas Island altered three trophic levels and led to shifts in the island's rainforest ecosystems from (B) an open to (C) a dense understory (89) [photo credit: P. T. Green]. Symbols are used courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (www.ian.umces.edu/symbols/).

but whether this pattern holds at other times in the past and whether it emerges on shorter time scales are unknown. Overall, knowledge about the temporal evolution of biotic interaction baselines would itself be highly informative and would also provide the foundation for assessing future changes in biotic interactions.

Another theme that is consistent through time is that novel biotic interactions often arise in rapidly changing environments (9, 22, 37, 77) and that dispersal may play a key role in mediating these changes in biotic interactions. Even though contemporary and fossil evidence shows that dispersal differences and biotic interactions can combine to mediate species' responses to climate change (23, 30, 53, 54), more research is needed to make explicit links between dispersal and biotic interactions through time. A first step toward this goal would be to examine patterns of species co-occurrence across space and time and determine to what extent the stability of those patterns differs between vagile and dispersal-limited taxa. Related, the geographic distributions of numerous taxa shifted substantially during the late Quaternary, and most studies have attributed these changes to individualistic responses governed primarily by environmental constraints (78, 79). However, for range shifts that are not fully explained by climate change, the extent to which the mismatch is due to dispersal limitation versus concerted responses stemming from biotic interactions [or both (53)] is unclear (30, 54).

The widely disparate observational time scales of the past and the recent present hinder full realization of these emerging insights (80), but this problem in part can be ameliorated by controlling for the amount of time across which rates of biotic and climate change are calculated (68). Although we lack direct knowledge of the detailed ecology of many extinct species, recent studies have shown that a focus on taxon-free metrics such as functional groups or traits can be informative in making comparisons across time intervals (34, 64). An important next step is to extend these efforts to the responses of biotic interactions to climate change across time scales. Similarly, metrics such as community or food web structure that are relatively independent of particular species can provide a "common currency" [(77); (81), p. 747] and framework for discussing future community and ecosystem changes that translate irrespective of time scales (82).

For all of these challenges, further study of the Quaternary record will be of paramount importance. The Quaternary fossil record serves a central role in bridging from the ecological time scales of the present to evolutionary scales seen in deep time. This record is data-rich, and for some systems or sites, time scales of change can be resolved to decades or less (83). Climate changes during this period are relatively well understood from independent evidence and models (84) and include multiple glacial-interglacial cy-

cles. Quaternary assemblages typically contain many extant species, and genetic and isotopic data are available for many species and assemblages (35, 78). Multiple lines of evidence can be used to evaluate the effects of specific climatic drivers on the structure of biotic interaction networks at multiple spatial and temporal scales. Comparisons between modern and Quaternary systems can help illuminate mechanisms and test the generality and permanence of short-term patterns [for example, by teasing apart the roles of climate, CO₂, and fire in functional shifts in vegetation type (41)]. Similarly, comparisons between the Quaternary and older intervals can test whether patterns observed on comparatively short time scales hold across longer intervals and elucidate the circumstances under which ecological changes translate into evolutionary change [for example, comparing current and expected future extinction rates to mass extinction events (10, 68)]. A detailed examination of the Quaternary fossil record will be key to integrating insights from fossil and extant systems and, ultimately, improving our ability to anticipate the effects of climate change on ecosystems in the future.

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REVIEW

The Future of Species Under Climate Change: Resilience or Decline?

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As climates change across already stressed ecosystems, there is no doubt that species will be affected, but to what extent and which will be most vulnerable remain uncertain. The fossil record suggests that most species persisted through past climate change, whereas forecasts of future impacts predict large-scale range reduction and extinction. Many species have altered range limits and phenotypes through 20th-century climate change, but responses are highly variable. The proximate causes of species decline relative to resilience remain largely obscure; however, recent examples of climate-associated species decline can help guide current management in parallel with ongoing research.

A better understanding of how species respond to ongoing anthropogenic climate change is crucial for assessing vulnerability and guiding efforts to avoid potentially severe biodiversity loss (1, 2). However, whereas forecasts of changes in species' geographic ranges typically predict severe declines (3, 4), paleoecological studies suggest resilience to past climatic warming (Fig. 1) (5–7). Superficially, it seems that either forecasts of future response are overestimating impacts (8) or that history is somehow an unreliable guide to the future (9). Here, we explore the apparent contradiction between (observed) past and (predicted) future species responses by first summarizing salient concepts and theory, then reviewing (i) broad-scale predictions of future response and (ii) evidence from paleontological and phylogeographic studies of

past responses at millennial or greater time scales. To bridge the two, we consider evidence for responses to more recent (20th-century) climate change. Finally, we place these observations in a management context.

What Theory Says: Concepts and Predictive Models

In principle, the vulnerability of a given species to climate change is a combination of exposure (that is, regional or “mesoscale” change in climatic means and extremes) and intrinsic sensitivity (for example, due to physiological limits, habitat or trophic specialization, life history characteristics, or obligate species interactions). These factors are mediated by response, defined as the capacity of local populations to buffer climatic alterations in situ via plastic reactions (including behavioral responses) or genetic adaptation, or by shifting geographically to track optimal conditions (Fig. 2A) (1, 2, 10).

Exposure is typically measured as shifts in mean precipitation or temperature at the mesoscale (e.g., 1 to 100 km²). For temperature, ensemble forecasts tend to predict the largest increases in northern high latitudes and the lowest across

the southern oceans (11). Novel climatic conditions, in which new species assemblages might form, are predicted for the tropics, with disappearing climates in the mountains (12). The expected increase in frequency of extreme climate events will probably also affect species persistence (13, 14). An important consideration here is how landscape features such as slope, aspect, vegetation cover, and soil moisture can ameliorate shifts in means and extremes of temperature at the microenvironmental scale that organisms actually experience (1, 15–19). In this context, topographically complex areas provide potential climate change refugia (microrefugia) (19–22), whereas low-relief topography can exacerbate climate change impacts, as organisms must move further to remain in the same climate space (23). In lowland areas, the requirement to move larger distances to track climate, especially if combined with dispersal limitation due to habitat fragmentation, can cause a lag in the response, possible leading to lowland biotic attrition with important changes in ecosystem functioning (24).

A key dimension of species' response is the capacity to persist in situ by altering fitness-related traits by plastic change or genetic adaptation. Plastic responses are undoubtedly important for short-term persistence (25, 26), but they can also entail costs (27) and may be insufficient to avoid extinction (28). Evolutionary rescue requires moderate-to-high heritability of key traits and/or high potential growth rates of populations, with critical levels of these parameters increasing with the rate of change (29–31) (Fig. 2B). All of the above is subject to fitness trade-offs across genetically correlated traits, which can further constrain evolutionary response (32). So far, and despite abundant evidence for adaptive variation across contemporary climatic gradients, direct evidence of genetically based adaptation to climate change over time remains sparse (33–36).

Perhaps the greatest potential for species to respond to climate change rests with local shifts in microhabitat use and dispersal to track suitable

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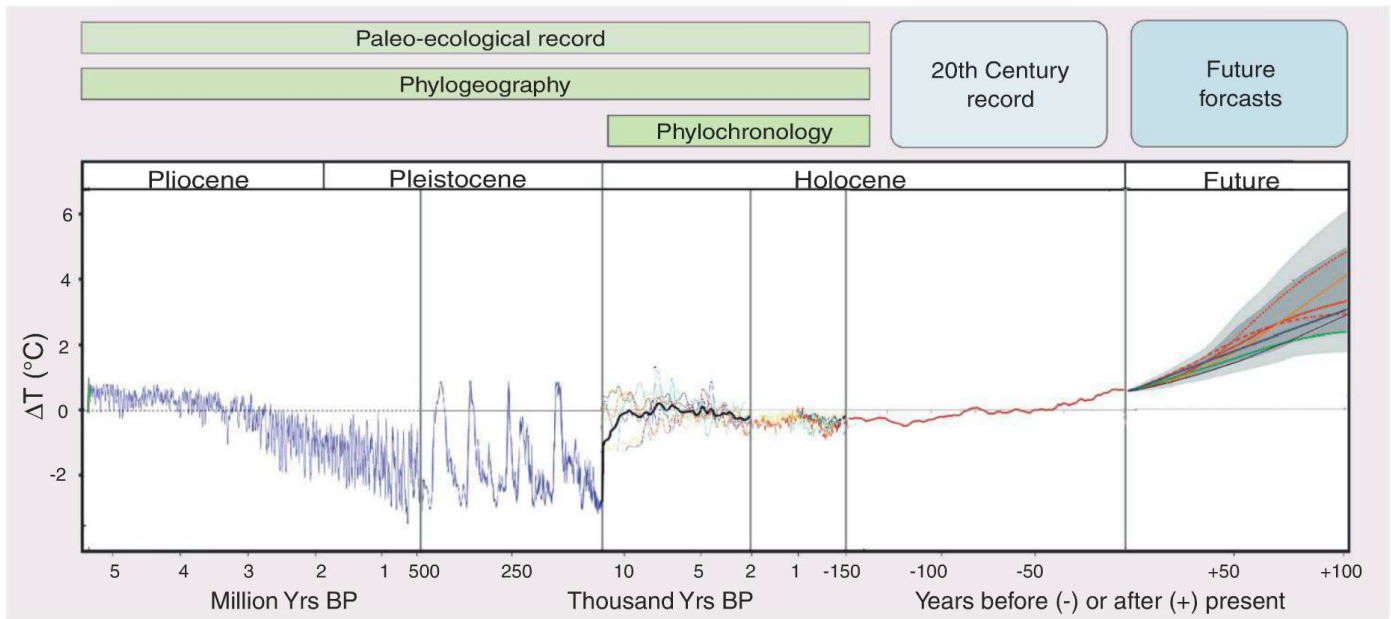


Fig. 1. Global mean temperature fluctuations and scales of inference across the historical record and future predictions. The paleoclimate record is modified from http://commons.wikimedia.org/wiki/File:All_palaeotemps.png, data for the 20th-century record were obtained from http://data.giss.nasa.gov/gistemp/graphs_v3/, and forecasts of future change are adapted

from (107), figure SPM.5 (different colors represent predictions under different models). Note the differences in scale on the x axis and that forecasts under higher-emission scenarios exceed the natural variability observed over the historical record. ΔT , change in temperature; Yrs BP, years before the present.

climatic conditions. Species that actively thermo-regulate may be able to select microhabitats that are buffered from extreme conditions (20, 37), though this can also restrict activity, which may lead to local extinction (38). This aspect of response to climate change has not been studied sufficiently and warrants greater attention. Dispersal to track geographic shifts in climate is clearly the dominant response measured from paleontological and 20th-century records (see below). The scale of dispersal required is a function of both the regional magnitude of climate change and topography, combined with the species sensitivity (23).

Predicted Impacts of Future Climate Change

Forecasts of potential species responses to future climate change come in two varieties: (i) correlative or mechanistic models of individual species (39) or (ii) prediction of higher-level properties such as species richness (3) or turnover (40). Correlative models are currently the most widespread and scalable method (41), but they have inherent limits. These models typically apply some form of climate envelope approach, assessing whether the (realized) climate niche occupied by a species continues to exist within the current geographic range and whether it will shift elsewhere or cease to exist. This approach has often been criticized for lacking a direct mechanistic basis and the inherent danger of extrapolation (9). Additionally, these models are generally computed at a coarse spatial resolution and fail to capture spatial variability in temperature over

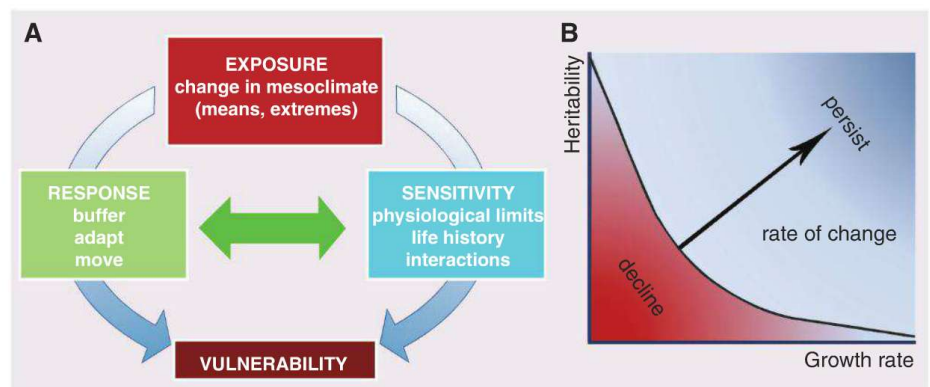


Fig. 2. Factors affecting species vulnerability to climate change. (A) Schematic of the pathway from exposure to broad-scale climate change to species vulnerability [see (1, 2) for analogous representations]. (B) Limits to evolutionary rescue imposed by trait heritability, the intrinsic rate of population growth, and the rate of change in the environment (e.g., temperature). This schematic is modified from (36) and is based on theoretical models in (29).

tens to hundreds of meters, at which the buffering role of microhabitat heterogeneity may be crucial for species persistence (18, 42). Thus, correlative models are probably a better measure of exposure than of species vulnerability to climate change.

The actual predictions of effects on species persistence are often dire, however. For example, one prominent analysis predicted that 15 to 37% of species would be endangered or extinct by 2050 (3). Another predicts more than a 50% loss of climatic range by 2080 for some 57% of widespread species of plants and 34% of animals (4). Montane taxa are expected to lose range area

as they shift upward with warming. Again, predicted effects are catastrophic (43–45) and could be even worse for the highly endemic biotas of tropical montane forests if the cloud base lifts (46). For the tropical lowlands, high levels of species attrition are predicted because of narrower physiological tolerances (47) and a high velocity of change due to shallow temperature gradients (48). Reduction of species ranges is expected to result in substantial loss of geographically structured genetic variation, perhaps including cryptic taxa (49, 50). Yet, we must acknowledge the level of uncertainty of these predictions and the possibility that these

models are overestimating extinction risk. Future models should be improved by incorporating key parameters such as finer-scale topographic heterogeneity (18), interaction of biotic (51, 52) and other anthropogenic factors (7, 45, 53), species physiological constraints and plastic acclimation capacity (39), as well as demographic processes [see for instance, the recent findings of Reed *et al.* (54) in a wild population in which density-dependent compensation counteracts the reduced fledgling rates due to phenological mismatch provoked by climate change].

What the Data Say: Species Responses to Past Climate Change

The Paleoeological Record

The fossil record and the imprint of history in geographic patterns of DNA diversity (phylogeography) provide valuable insights into how species responded to past shifts in global temperature, including rapid warming events at the Pleistocene-Holocene transition (Fig. 1). These sources of information on historical responses have distinct limitations that can be partially overcome by combining types of evidence (see below). The fossil record varies in extent and resolution according to preservation conditions (55, 56); that is, a sparse faunal record for the tropics; underrepresentation of small, rare, and physically fragile species; and, sometimes, low taxonomic resolution (i.e., identification to genus rather than species). Phylogeographic analysis, on the other hand, affords higher spatial resolution but typically has low temporal precision compared with fossils.

The picture emerging from fossil evidence, including the Pleistocene-Holocene transition, is one of both robustness and dynamism. To simplify, there was no signal of elevated extinction through periods of rapid change (5, 6, 57), and, at the level of genera, composition and trophic

structure of mammalian communities appear robust [(5), but see (58)]. One exception is recent megafaunal extinctions, where climate change and human impacts likely combined with devastating consequences (59, 60). This is not to say that the biota was static through past climate change—far from it. The dominant response was idiosyncratic shifts in geographic range (61–63) with concomitant shuffling of community composition, often resulting in nonanalog assemblages (9). Geographic shifts are well described for mammals and appear more pronounced for habitat or dietary specialists than generalists (5, 64, 65). Another type of response described well in mammals through past warming periods is decrease in body size, a key ecological trait (5, 66).

Comparative phylogeographic studies, often combined with paleoclimatic modeling of geographic ranges, offer another window on past species responses (67) and can identify regions in which taxa persisted through past climate change; that is, evolutionary refugia (68–70). Again, such studies point to disparate species' responses, with some evidently persisting in many areas and others in just a few major refugia, despite a common history of climate change across the focal landscape (71–74). When combined with fossil evidence and spatial models, such studies highlight the extent of range shifts but also the importance of scattered microrefugia, which are important for range recovery (6, 75) and perhaps also harbor distinct adaptations (76, 77). Going further, direct DNA analyses of subfossils provide a much clearer picture of population dynamics through climate change (78) and, for megafauna, highlight differences among species in response to the twin challenges of climate change and human colonization (7, 79).

The 20th-Century Record

The discord between predictions of high extinction under future climate change and relatively

high resilience through paleoclimatic change could be partly due to the limitations of the fossil record (see above) but may also reflect the fact that, with the possible exception of Holocene megafauna, species were previously able to respond in the absence of other human-caused impacts on natural systems. Thus, even though the rate of expected future change may be much faster than that over the past century, there is value in examining how species have responded to climate change over the 20th century.

There is abundant evidence for climate-related changes in distributions and timing of life history events of species over the past decades. Meta-analyses across thousands of species report strong trends in shifts of geographic range limits, predominantly toward higher latitudes and higher elevations for terrestrial taxa and lower depths for marine taxa, as expected in a warming world (80–82). These trends are reflected even in increasing representation of more tropical species in major fisheries (83). Recent climate change has also affected the communities' composition by increasing the dominance of generalist taxa and larger basal prey species, whereas habitat specialists, rare species, and species with more northerly distributions have declined (84–87).

Yet again, a dominant feature is marked heterogeneity of species responses. For example, Chen *et al.* (80) report that about one-quarter of species moved downhill or toward lower latitudes, opposite of what was expected. This observation may reflect marked differences in 20th-century climate change across regions and between marine and terrestrial systems (88). However, the same is seen within a single biome [e.g., UK terrestrial species (80)]. To take one example, studies across strong environmental gradients in California revealed both upward and downward shifts in plants (89) and birds (90), whereas montane small mammals mostly shifted upward, in accord with increasing minimum temperatures and lead-

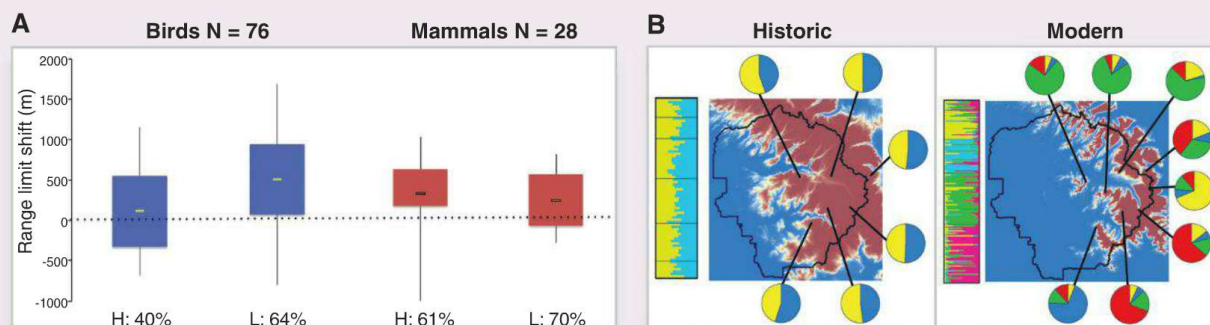


Fig. 3. Species responses to 20th-century climate change. (A) Examples of heterogeneity of shifts in range limits for small mammals and birds across Yosemite National Park, California, and across a century of environmental change. H, lower limit of high-elevation species; L, upper limit of low-elevation species; both are displayed with the percentage of species showing significant

range shifts. Data are from (90, 91). **(B)** Decreased extent and increased fragmentation of the range of the alpine chipmunk (*Tamias alpinus*) across Yosemite, with a concomitant increase in genetic structure associated with the upward contraction of this montane specialist from the early 20th century to the present. Modified from (108).

ing to substantial range contractions (91) (Fig. 3). Yet even closely related species (e.g., different species of chipmunk, voles, or field mice) showed disparate responses. Lenoir *et al.* (92) summarize some of these examples and suggest habitat modification, as well as species interactions and their interplay with climate change, as possible mechanisms explaining the observed variability. These observations highlight the complexity of the process and the difficulty of accurately predicting future effects based on actual models. This points to the need for a more nuanced approach to predicting species vulnerability—one that also considers changes in precipitation, productivity, and habitat structure (89, 90). It is difficult to identify traits that predict whether or not species will track temperature change (93). Species expanding ranges upward or to higher latitudes tend to be weedy, prolific, and/or ecological generalists (86, 87, 91, 94). But as yet, few, if any, traits provide robust prediction of which species are observed to contract in range. It is the latter we should be most concerned about.

Shifts in phenology (e.g., earlier flowering, breeding, and migration and reduced migration) are also widely observed in the 20th-century record and could cause temporal mismatch between strongly interacting species, especially where these species employ different environmental cues (28, 95, 96). As expected with warming, decreasing body size has been observed in several studies of birds and mammals (97). This response seems to be plastic rather than genetic (98, 99), or it may be related to extended food availability rather than direct physiological effects (100). Again, idiosyncrasy is the trend; some hibernating mammals show increasing body size, perhaps due to a longer period of food availability (100, 101). Finally, as yet, no species extinctions are clearly attributable to climate change per se, although several studies recorded local extinctions and population declines (102). Nevertheless, it is very difficult to establish causative relationships between warming and population declines or extinction, due to the interaction with other anthropogenic factors such as habitat loss or previously unseen pathogens [e.g., declines of amphibians in the montane neotropics (103, 104)]. A recurring message is that we have insufficient knowledge of the proximate cause(s) of observed species declines under global warming: The few examples appear to be more closely related to indirect ecological effects than to demonstrable physiological challenges (102).

Management in the Face of Change and Uncertainty

The historical record over millennia and the past century demonstrates that species do respond to climate change, albeit in ways difficult to predict individually. As we move into climate conditions without recent parallel and across ecosystems already strongly affected by humans, the challenge is to increase resilience of natural systems now, in

conjunction with continuing research to improve our capacity to predict vulnerability (1, 2). These priorities must undoubtedly be accompanied by the urgent mitigation of the main culprit, the greenhouse gas emissions (4).

What Do We Know?

The simplest and most strongly supported response of species is to shift geographically to track their climatic niche. Observed responses to paleoclimatic change emphasize the importance of refugia—both macro- and microrefugia (16)—as key landscapes to protect.

Given rapid climatic change, evolutionary rescue of intrinsically sensitive species is most plausible for those with short generation times and high potential population growth. In particular, for potentially sensitive species with long generation times, every effort should be made to minimize other stressors on population viability and to monitor population trends.

Taken together, managing and restoring evolutionary dynamics across large ecologically heterogeneous landscapes, including long-term climatic refugia, and enabling habitat connections to these refugia are increasingly acknowledged as priorities. Recognizing that species and ecosystems are naturally dynamic and are likely to become more so with anthropogenic impacts, maintaining the status quo should not be the conservation goal; rather, we should seek to manage system dynamics within bounds to avoid large-scale state changes (105, 106).

What Don't We Know? Some Research Priorities

Understanding and predicting the effects of future climate change on species, let alone communities and ecosystems, is an urgent and fundamental challenge to this generation of biologists. Although we have identified many areas of uncertainty and more can be found in the broader literature, we will now highlight just three areas of immediate relevance to conservation decision-makers.

First, understanding the capacity of species to buffer effects of climate change *in situ* is crucial if we are to predict and manage vulnerable species. Key aspects include better understanding of the limits of plasticity of key traits and microhabitat buffering. Along the same lines, aside from some generalizations, research on trait-based prediction of vulnerability has a long way to go before it can provide a robust management tool. Progress on these aspects will come from intensive analyses of the proximate causes of climate-related species decline, as well as further comparative studies.

Second, predictive models of spatial and demographic responses of species must be tested and improved, yet must also remain scalable to many species. Parameter-rich models incorporating demography, dispersal, intrinsic limits, and evolutionary response are ideal and can be applied to model systems. The identification of generalizations and hybrid approaches will enable

more robust predictions for larger numbers of less well known species.

Third is the vexing problem of species interactions: Do tipping points exist and lead to irreversible state change (52, 105)? How do we reconcile these concerns with evidence for dynamism of communities and resilience of trophic structure through past climate change?

Underpinning all of the above is the need to make greater use of the record of the responses to past climate change, over time scales from millennia to decades. The potential of museums and herbaria collections and records is becoming more apparent, but much more needs to be done to capture and apply the invaluable data and field notes from long-term studies of 20th-century ecologists.

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REVIEW

Climate Change Impacts on Global Food Security

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Climate change could potentially interrupt progress toward a world without hunger. A robust and coherent global pattern is discernible of the impacts of climate change on crop productivity that could have consequences for food availability. The stability of whole food systems may be at risk under climate change because of short-term variability in supply. However, the potential impact is less clear at regional scales, but it is likely that climate variability and change will exacerbate food insecurity in areas currently vulnerable to hunger and undernutrition. Likewise, it can be anticipated that food access and utilization will be affected indirectly via collateral effects on household and individual incomes, and food utilization could be impaired by loss of access to drinking water and damage to health. The evidence supports the need for considerable investment in adaptation and mitigation actions toward a “climate-smart food system” that is more resilient to climate change influences on food security.

Tackling hunger is one of the greatest challenges of our time (1). Hunger has multi-dimensional dimensions and causes, ranging from

deficiencies in macro- and micro-nutrients, through short-term shocks on food access, to chronic shortages. Causes range from constraints on the supply

of food of sufficient quantity and quality and lack of purchasing power to complex interactions of nutrition with sanitation and infectious diseases leading to poor health. Several of these causes have been addressed in recent decades, and substantial progress has been made in reducing the proportion of the world's undernourished population from an estimated 980 million in 1990–92 to about 850 million in 2010–12 (2). However, from other relevant indicators of nutrition, such as child underweight and stunting and health surveys, an estimated 2 billion people still suffer from micro-nutrient deficiencies today.

The long-term reduction in the prevalence of undernutrition worldwide has slowed since 2007, as a result of pressures on food prices, economic

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volatilities, extreme climatic events, and changes in diet, among other factors. Furthermore, additional pressures on the global food system are expected to build in the future. For example, demand for agricultural products is estimated to increase by about 50% by 2030 as the global population increases (3), which will require a shift toward sustainable intensification of food systems (4). The impacts of climate change will have many effects on the global food equation, both for supply and demand, and on food systems at local levels where small farm communities often depend on local and their own production (5). Thus, climate change could potentially slow down or reverse progress toward a world without hunger.

Here, we offer an overview of the evidence for how climate change could affect global food security, with particular emphasis on the poorer parts of the world. We deliberately take a broad view of the complex interactions between climate change and global food security, stating what we do know with some degree of confidence, as well as acknowledging aspects where there is little or no evidence. We end by proposing a number of precepts for those making policy or practical decisions on climate change impacts and food security.

Food Security

Together, climate change and food security have multiple interrelated risks and uncertainties for societies and ecologies. The complexity of global

food security is illustrated by the United Nations' Food and Agricultural Organization (FAO) (6) definition: (i) the availability of sufficient quantities of food of appropriate quality, supplied through domestic production or imports; (ii) access by individuals to adequate resources (entitlements) for acquiring appropriate foods for a nutritious diet; (iii) utilization of food through adequate diet, clean water, sanitation, and health care to reach a state of nutritional well-being where all physiological needs are met; and (iv) stability, because to be food secure, a population, household or individual must have access to adequate food at all times.

It is extremely challenging to assess precisely the current status of global food security from such a broad concept. However, the big picture is clear: About 2 billion of the global population of over 7 billion are food insecure because they fall short of one or several of FAO's dimensions of food security. Enormous geographic differences in the prevalence of hunger exist within this global estimate, with almost all countries in the most extreme "alarming" category situated in sub-Saharan Africa or South Asia (7) (Fig. 1).

Nevertheless, it is important to note that the current numbers for undernourished people are rough estimates at best and are seriously deficient in capturing the access, utilization, and stability dimensions of food security. First, the methods used to make these estimates only cap-

ture longer-term trends, not the short-term changes that can be an important consequence of climate variability. The most recent data are averages for the period 2010–2012 (2), so they do not capture a specific year, let alone shorter-term shocks, be they climate-related or otherwise. Second, they estimate calorie shortage only and do not cover other dietary deficiencies and related health effects that can impair physical and mental capacities. Third, they are derived from aggregate data, not actual household or individual-level food deficiencies, which hinders analyses of distributional effects of climate and other shocks. The FAO methodology was recently improved (8), but the above shortcomings could not be addressed within the framework of the current method, and thus, current analyses of climate change impacts on food security are incomplete. An overhaul of data-gathering methods that encompasses food deficiencies at household levels, as well as nutritional status, is needed.

Climate Change

There is a substantial body of evidence that shows that Earth has warmed since the middle of the 19th century (9–14). Global mean temperature has risen by 0.8°C since the 1850s, with the warming trend seen in three independent temperature records taken over land and seas and in ocean surface water (15). Climate change can result from natural causes, from human activities

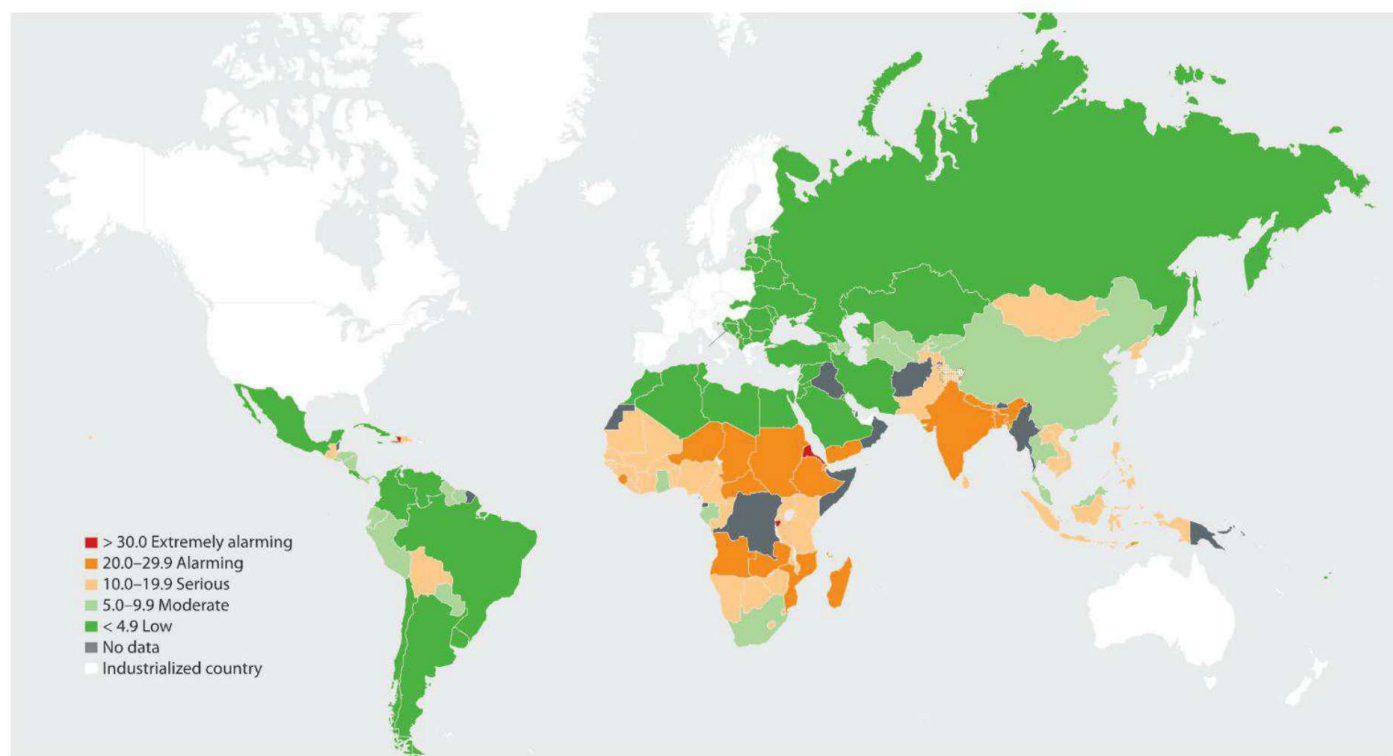


Fig. 1. Global distribution of hunger as quantified by the 2012 Global Hunger Index. The Welthungerhilfe, IFPRI, and Concern Worldwide Hunger map 2012 calculated a Global Hunger Index (7) for 120 countries by using the proportion of

people who are undernourished, the proportion of children under 5 who are underweight, and the mortality rate of children younger than age 5, weighted equally. [Reproduced with permission from Welthungerhilfe, IFPRI, and Concern Worldwide (7)]

through the emission of greenhouse gases such as carbon dioxide and methane, and from changes in land use. Carbon dioxide (CO₂) levels in the atmosphere have increased from about 284 ppm in 1832 to 397 ppm in 2013 (16), and there is a theoretical link between the levels of such “greenhouse” gases in the atmosphere and global warming. Three independent reviews have found strong evidence for human causes for the observed temperature warming mainly caused by the burning of fossil fuels, with smaller contributions from land-use changes (15–18).

Thus, climate change is expected to bring warmer temperatures; changes to rainfall patterns; and increased frequency, and perhaps severity, of extreme weather. By the end of this century, the global mean temperature could be 1.8° to 4.0°C warmer than at the end of the previous century (15). Warming will not be even across the globe and is likely to be greater over land compared with oceans, toward the poles, and in arid regions (15). Recent weather records also show that land surface temperatures may be increasing more slowly than expected from climate models, potentially because of a higher level of absorption of CO₂ by deep oceans (19). Sea-level rises will increase the risk of flooding of agricultural land in coastal regions. Changes in rainfall patterns, particularly over tropical land, are less certain, partly because of the inability of the current models to represent the global hydrological cycle accurately (20). In general, it is expected that the summer Asian monsoon rainfall may increase, while parts of North and southern Africa could become drier (15). How will these regional changes in climate affect food security?

Research Biases

Agriculture is inherently sensitive to climate variability and change, as a result of either natural causes or human activities. Climate change caused by emissions of greenhouse gases is expected to directly influence crop production systems for food, feed, or fodder; to affect livestock health; and to alter the pattern and balance of trade of food and food products. These impacts will vary with the degree of warming and associated changes in rainfall patterns, as well as from one location to another.

Climate change could have a range of direct and indirect effects on all four dimensions of food security. How is the evidence base distributed across the dimensions of food security? We undertook a bibliographic analysis of peer-reviewed journal papers on food security and climate change since the publication of the first Intergovernmental Panel on Climate Change (IPCC) report in 1990 (21). That report was ground-breaking for the climate science that it reviewed, but agriculture was entirely absent. Our analysis shows that a small peak of papers with climate change and food security in the title or abstract were published in the mid-1990s, followed by a lull then a sharp increase in papers published with these terms from 2008 onward.

The distribution of the evidence across the four dimensions of food security is, however, heavily skewed toward food availability within 70% of the publications. Access, utilization, and stability dimensions of food security are represented by only 11.9, 13.9, and 4.2% of the total publications on food security and climate change, respectively.

Why is the evidence based on climate change impacts so unevenly distributed across the four dimensions of food security? There are several possibilities. Research has largely concentrated on the direct effects of climate change, such as those on crop growth and on the distribution of agricultural pests and diseases. Also, studies have understandably focused on areas that can be easily investigated, often through analyzing single-factor changes, and have avoided the com-

plex and multilayered features of food security that require integrations of biophysical, economic, and social factors. Clearly, current knowledge of food security impacts of climate change is dramatically lacking in coverage across all dimensions of food security. Nevertheless, where there is good evidence, what are the broad conclusions?

Food Availability

Rosenzweig and Parry (22) produced the first global assessment of the potential impacts of climate change scenarios on crops. They used numerical crop models of wheat, rice, maize, and soybeans to simulate yields at 112 locations in 18 countries, in the current climate and under climate change using the output of three climate models. These point-based estimates of change

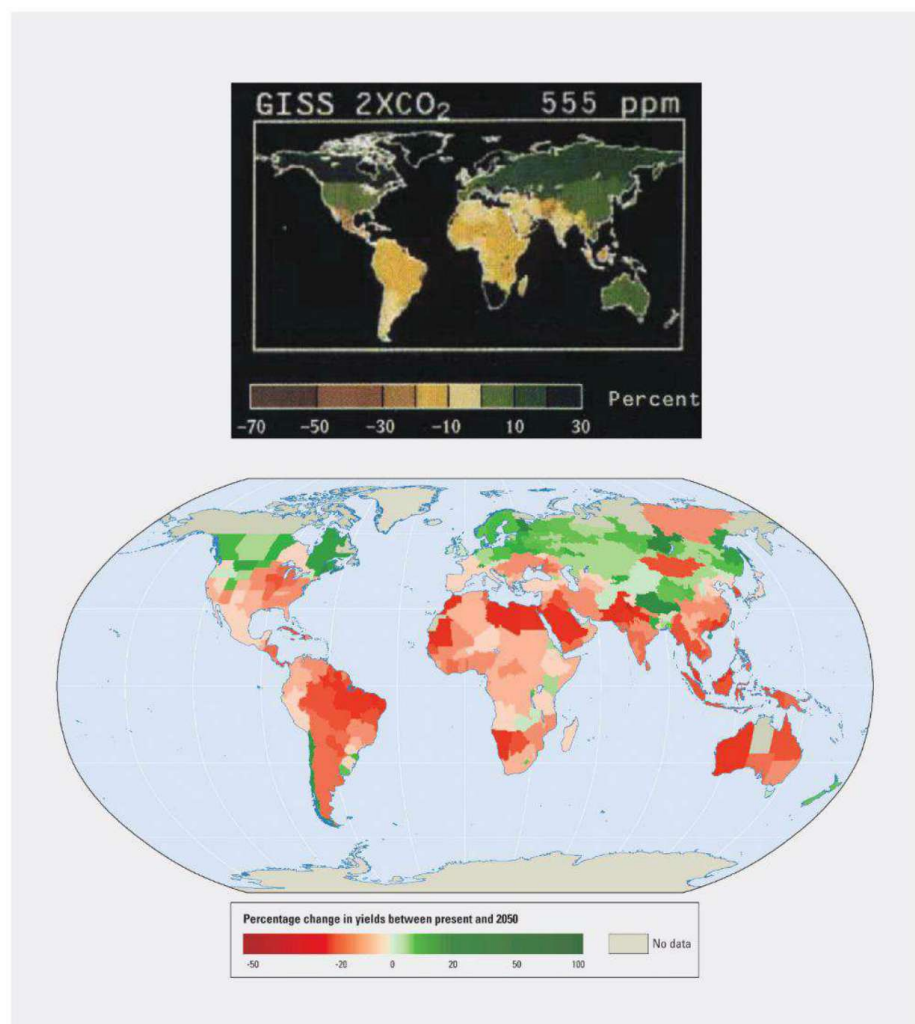


Fig. 2. Global impacts of climate change on crop productivity from simulations published in 1994 and 2010. (Top) The 1994 study (22) used output from the GISS GCM (in this example) with twice the baseline atmospheric CO₂ equivalent concentrations as input to crop models for wheat, maize, soybean, and rice that were run at 112 sites in 18 countries. Crop model outputs were aggregated to a national level using production statistics. **(Bottom)** The 2010 study (27) simulated changes in yields of 11 crops for the year 2050, averaged across three greenhouse emission scenarios and five GCMs. [Reprinted by permission from (top) Macmillan Publishers Ltd. (22); (bottom) World Bank Publishers (27)]

were then scaled-up to country level by using national crop production statistics. Future climate simulations under both present-day and doubled- CO_2 concentrations were used. They found that enhanced concentrations of atmospheric CO_2 increase the productivity of most crops through increasing the rate of leaf photosynthesis and improving the efficiency of water use. However, more recent research has proposed that the CO_2 yield enhancement in crop models is too large compared with observations of crop experiments under field conditions (23). If true, these revised estimates will affect the magnitude of the previous global crop yield changes but not the spatial distribution of impacts. Even if there is some debate on the magnitude of CO_2 effects, higher concentrations of CO_2 in the atmosphere are already having noticeable continental level effects on plant growth in sub-Saharan Africa (24).

The simulations of Rosenzweig and Parry (22) showed that there is a large degree of spatial variation in crop yields across the globe. Both the sign and magnitude of the projected changes in crop yield vary with alternative climate models and from one country to another. In general, yields increased in Northern Europe, but decreased across Africa and South America (22) (Fig. 2). Inevitably, there were methodological weaknesses in this study, including the use of only just over one hundred points to represent global crop production, the absence of any change in the areas suitable for crop production in future climates, limitations on how each of the model points is representative of their surrounding regions, and assumptions about varieties in the crop model parameters themselves. Nevertheless, as the first example of global impacts of climate change on crop production, these simulations are remarkable.

Since 1994, knowledge of the effects of climate on crop plant physiology has improved, the skill of simulation methods for climate change impact studies has increased, and better computing power and data sets to run global simulations have become available. Landmark studies since 1994 include those by Parry and colleagues (25), Cline (26), and, most recently, the World Bank (27) (Fig. 2). Specific projections vary with the climate model scenario used, the simulations methods, and the time scale over which the projections are done. However, the broad-scale pattern of climate change impacts on crop productivity and production has remained consistent across all of these global studies spanning almost 20 years of research. Crop yields are more negatively affected across most tropical areas than at higher latitudes, and impacts become more severe with an increasing degree of climate change. Furthermore, large parts of the world where crop productivity is expected to decline under climate change (Fig. 2) coincide with countries that currently have a high burden of hunger (Fig. 1). We conclude that there is a robust and coherent pattern on a global scale of the impacts of climate change

on crop productivity and, hence, on food availability and that climate change will exacerbate food insecurity in areas that already currently have a high prevalence of hunger and undernutrition.

A recent systematic review of changes in the yields of the major crops grown across Africa and South Asia under climate change found that average crop yields may decline across both regions by 8% by the 2050s (28). Across Africa, yields are predicted to change by -17% (wheat), -5% (maize), -15% (sorghum), and -10% (millet) and, across South Asia, by -16% (maize) and -11% (sorghum) under climate change. No mean change in yield was detected for rice. Knox *et al.* (28) concluded that evidence for the impact of climate change on crop productivity in Africa and South Asia is robust for wheat, maize, sorghum, and millet, and inconclusive, absent, or contradictory for rice, cassava, and sugarcane.

Global-scale climate change impacts at a grid scale of 200 to 250 km can provide useful information on shifts in production zones and perhaps guide the focus of global crop improvement programs seeking to develop better-adapted crop varieties. However, much of the adaptation of agricultural practice to climate change will be driven by decisions at the farm and farm-enterprise scale. These decisions need much finer resolution information than that shown in Fig. 2. At much finer grid scales of 5 to 20 km there are even greater limits to the skill of predictive crop science than at the global scale. Additional uncertainties arise from how the output of global-scale climate models is down-scaled, whether input data (such as crop, soil, topographic, and management information) are available across the domain for crop simulation at this scale, as well as questions as to how skillful the simulation methods are across a fine-scale domain. Recent attempts to harmonize modeling approaches for wheat simulations under climate change found considerable variation in projected impacts among models owing to differences in model structures and parameter values (29). It is not surprising that the sheer complexity of food production systems at a very fine scale is difficult to reproduce in numerical models. However, there is a real need for studies that test how well fine-scale simulations compare with observations in the current climate, as a necessary test of their utility in future climates.

Although the evidence for direct climate change effects on crop productivity is reasonable, important limitations remain for impacts on food availability more broadly. First, models that adequately capture expected climate change effects on crops are only available for the major cereals, groundnut, and some roots and tubers. Impacts on other important crops (such as vegetables, pulses, and locally important, but globally minor, crops) are often inferred based on similar plant characteristics, rather than studied explicitly. Second, changes in grassland productivity and grazing quality and the quality of crops for livestock feed (30) have hardly

been captured, which limits the understanding of climate change–livestock linkages. Last, many crop studies capture the impacts of mean changes in climate, but are less accurate for changes in weather extremes, which can have even more important consequences for crop yields (31).

Food Access

Food access (and utilization) connects to climate change through indirect, but well-known, pathways. Access to food is largely a matter of household and individual-level income and of capabilities and rights. Food access issues have been studied through two types of approaches: top-down by models that attempt to link macro-shocks to household level responses and adaptation outcomes; and by community- and household-level studies that try to assess climate change effects from the bottom up.

The macro-models are often composed of interlinked models—including climate, crop, and economic models. In this approach, outcomes from a climate model feed into the crop model to simulate crop yields under different climate scenarios. The simulated yields are then used to make economic forecasts for the impact of climate change on prices, incomes, trade, and such like. The macro-models can either be constructed following a partial equilibrium approach, i.e., studying the impacts only in one specific sector, such as agriculture, or as general equilibrium models seeking to capture the impacts on the whole economy. The weakness of this approach is that it barely captures climate adaptations. In contrast, micro-level studies are often based on detailed household surveys and usually better account for adaptation by households and communities to climate change.

An important example is the International Food Policy Research Institute (IFPRI) International Model for Policy Analysis of Agricultural Commodities and Trade (IMPACT) model, which connects climate change scenarios with food supply effects and market and price outcomes, and traces the economic consequences of food availability drivers to access and utilization of food, that is, food energy consumption and children's nutritional situation (32, 33). Specific findings are heavily dependent on the assumptions made about future income and population growth but, in general, show clear linkages between economic growth and resilience to climate change (32).

A host of studies is emerging that analyzes what happens to communities and households when they are exposed to climate shocks (34–37). These approaches tend to capture more adaptation capabilities than macro-models, such as asset draw-down, job-switching, migration, social policy responses, and collective action for adaptation and assistance. But it is difficult to appropriately capture with micro-level studies the covariate risks of climate change that cut across broad regions.

Climate change could transform the ability to produce certain products at regional and international levels. If it turns out, for example, that the



geography of biomass production shifts at a global scale (38), this will have production implications for all bio-based products—whether food, feed, fuels, or fiber—and will impinge on food trade flows, with implications for (farm) incomes and access to food (39). Similar changes have been observed in the geography and relative productivity of certain ocean species, such as shifts between anchovy and sardine regimes in the Pacific Ocean (40).

Thus, macro-modeling and micro-level analyses of climate change linkages to food security are complementary. The prices of the basic resources, such as land and water, are formed by long-term expectations (41, 42), and these prices encompass expectations of climate change, such as revaluation of land with access to water. Structural consequences can emerge, particularly when property rights are lacking and traditional land and water rights are not protected, as is the case in many developing countries with food security problems (43–45); these structural problems lead to erosion of the assets of the poor, as seen during “land grabbing” by external and foreign interests (46).

Food Utilization

Food utilization, to attain nutritional well-being, depends upon water and sanitation and will be affected by any impact of climate change on the health environment. Little research has been done on this dimension of food and nutrition security. Links with drinking water may be obvious, when climate variability stresses clean drinking water availability (47, 48). Hygiene may also be affected by extreme weather events causing flooding or drought in environments where sound sanitation is absent (49–51). In addition, uptake of micronutrients is adversely affected by the prevalence of diarrheal diseases, which in turn is strongly correlated with temperature (52).

Climate change can also impinge on diet quality, and increased costs may result from measures required to avoid food contamination stemming from ecological shifts of pests and diseases of stored crops or food (53, 54). Science and innovation have a role to play here, and in recent years there has been good progress made in improving food utilization through fortification and biofortification (55, 56). Vulnerability to food security shocks needs further research, as do ways to strengthen adaptive capacities under climate change, (57) as public policy responses depend on such insights. For example, appropriate design of programs transferring income to the poor, employment-related transfer programs, and early childhood nutrition actions (58–60) may all need expanding to respond to climate-related volatilities.

New nutritional stresses are emerging, and the most striking example has been the recent “nutrition transition,” i.e., the process by which globalization, urbanization, and changes in lifestyle are linked to excess caloric intake, poor-quality diets, and low physical activity. Together, these factors have led to rapid rises in the inci-

dence of obesity and chronic diseases, even among the poor, in developing countries (61). The nutrition transition will unfold in parallel with climate change in coming decades, but very little research on the potentially reinforcing effects of these phenomena has been done.

Stability of the Food System

The stability of whole food systems may be at risk under climate change, as climate can be an important determinant for future price trends (32), as well as the short-term variability of prices. Since 2007, the world food equation has been at a precariously low level and, consequently, even small shocks on the supply or demand side of the equation will have large impacts on prices, as experienced in 2008 (62). Food security of the poor is strongly affected by staple food prices, as a large part of an impoverished family’s income has to be spent on staple foods.

Climate change is likely to increase food market volatility for both production and supply [see (63) for the supply side]. Food system stability can also be endangered by demand shocks, for instance, when aggressive bioenergy subsidies and quota policies were applied by the political economy (64). These sorts of policy shifts, made in the past decade by the United States and the European Union, have been motivated in part by energy security concerns and partly by climate mitigation objectives (65–67). The resulting destabilization of food markets, which contributed to major food security problems, was therefore partly related to climate change (policy).

The 2008 food crisis stemmed from a combination of a general reduction of agricultural productivity and acute policy failures, exacerbated by export restrictions applied by many countries, a lack of transparency in markets, and poor regulation of financial engagement in food commodity markets (68, 69). A broad set of risks needs to be considered, of which climate change is an increasingly important one, that can ripple out to destabilize food systems, resulting in high and volatile food prices that temporarily limit poor people’s food consumption (70–73), financial and economic shocks that lead to job loss and credit constraints (74), and risks that political disruptions and failed political systems cause food insecurity (75). This complex system of risks can assume a variety of patterns that could potentially collide in catastrophic combinations.

What We Need to Know—Research and Evidence Gaps

Despite a burgeoning literature over the past 5 years or so, much remains unknown about many food security impacts of climate change. Getting better evidence will help to some extent. For example, uncertainties in understanding the underlying science, social science, and economics of climate change impacts will reduce as the evidence base expands with more research. How-

ever, other uncertainties will always remain as they arise from projections of climate change, sources of natural variability in climate, and future pathways of emissions of greenhouse gases.

Four broad priorities for future research emerge from our review: (i) gathering evidence on the effects of climate change impacts on the food access, utilization, and stability dimensions in order to achieve a more holistic understanding of food security; (ii) understanding the indirect impacts of climate change on food security requires more comprehensive analytical approaches and sophisticated modeling, including links to the political economy; (iii) improving projections of regional climate change effects on food security at country level and on smaller scales that are crucial for decision-making for adaptation of food systems; (iv) better integrating of human dimensions of climate change impacts into food security planning—because food systems are ultimately driven by people and their behavioural responses to real and perceived changes in their local climate—that will be central to the adaptation to climate change and actions to address hunger.

What We Know We Know—Messages for Decision-Makers

Decisions still need to be taken by policy-makers and practitioners confronted with the prospect of climate change impacts on food security, despite very real uncertainties in current knowledge and future trends. For those making decisions, we propose, with a fair degree of confidence from the existing evidence, six precepts for the impacts of climate change on food security:

- 1) Climate change impacts on food security will be worst in countries already suffering high levels of hunger and will worsen over time.
- 2) The consequences for global undernutrition and malnutrition of doing nothing in response to climate change are potentially large and will increase over time.
- 3) Food inequalities will increase, from local to global levels, because the degree of climate change and the extent of its effects on people will differ from one part of the world to another, from one community to the next, and between rural and urban areas.
- 4) People and communities who are vulnerable to the effects of extreme weather now will become more vulnerable in the future and less resilient to climate shocks.
- 5) There is a commitment to climate change of 20 to 30 years into the future as a result of past emissions of greenhouse gases that necessitates immediate adaptation actions to address global food insecurity over the next two to three decades.
- 6) Extreme weather events are likely to become more frequent in the future and will increase risks and uncertainties within the global food system.

All of these precepts support the need for considerable investment in adaptation and mit-

igation actions to prevent the impacts of climate change from slowing progress in eradicating global hunger and undernutrition. A wide range of potential adaptation and resilience options exist and more are being developed. These need to address food security in its broadest sense and to be integrated into the development of agriculture worldwide. Building agricultural resilience, or “climate-smart agriculture,” through improvements in technology and management systems is a key part of this, but will not be sufficient on its own to achieve global food security. The whole food system needs to adjust to climate change, with strong attention also to trade, stocks, and to nutrition and social policy options. We need to work toward what could be termed a climate-smart food system that addresses climate change impacts on all dimensions of food security.

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REVIEW

Climate Change and Infectious Diseases: From Evidence to a Predictive Framework

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Scientists have long predicted large-scale responses of infectious diseases to climate change, giving rise to a polarizing debate, especially concerning human pathogens for which socioeconomic drivers and control measures can limit the detection of climate-mediated changes. Climate change has already increased the occurrence of diseases in some natural and agricultural systems, but in many cases, outcomes depend on the form of climate change and details of the host-pathogen system. In this review, we highlight research progress and gaps that have emerged during the past decade and develop a predictive framework that integrates knowledge from ecophysiology and community ecology with modeling approaches. Future work must continue to anticipate and monitor pathogen biodiversity and disease trends in natural ecosystems and identify opportunities to mitigate the impacts of climate-driven disease emergence.

The life cycles and transmission of many infectious agents—including those causing disease in humans, agricultural systems, and free-living animals and plants—are inextricably tied to climate (1, 2). Over the past decade, climate warming has already caused profound and often complex changes in the prevalence or severity of some infectious diseases (Fig. 1) (2–5). For human diseases, vector-control, antimicrobial treatments, and infrastructural changes can dampen or mask climate effects. Wildlife and plant diseases are generally less influenced by these control measures, making the climate signal easier to detect (4). For example, although the effects of climate warming on the dynamics of human malaria are debated, climate warming is consistently shown to increase the intensity and/or latitudinal and altitudinal range of avian malaria in wild birds (6, 7).

Predicting the consequences of climate change for infectious disease severity and distributions remains a persistent challenge surrounded by much controversy, particularly for vector-borne infections of humans [boxes S1 and S2 (8)]. Work using climate-based envelope models has predicted that modest climate-induced range expansions of human malaria in some areas will be offset by range contractions in other locations (9). An alternative approach, based on mechanistic

models of physiological and demographic processes of vectors and pathogens (10), predicts large geographic range expansions of human malaria into higher latitudes (11). Both approaches have their limitations (2), and the challenge remains to accurately capture the contributions of multiple, interacting, and often nonlinear underlying responses of host, pathogen, and vector to climate. This challenge is further exacerbated by variation in the climate responses among host-pathogen systems arising from different life history characteristics and thermal niches (12).

A decade ago, Harvell *et al.* (1) reviewed the potential for infectious diseases to increase with climate warming. Since then, the frequency of studies examining climate-disease interactions has continued to increase (Fig. 2), producing clear evidence that changes in mean temperature or climate variability can alter disease risk. Some of the best examples of climate responses of infectious diseases to date are from ectothermic hosts and from parasites with environmental transmission stages that can persist outside the host (Fig. 1). Indeed, first principles suggest that the rates of replication, development, and transmission of these pathogens should depend more strongly on temperature relative to other host-pathogen interactions. The next challenges require integrating theoretical, observational, and experimental approaches to better predict the direction and magnitude of changes in disease risk. Identifying the contribution of other environmental variables, such as precipitation, humidity, and climate variability remains a challenge (13, 14).

Here, we review the individual, community, and landscape-level mechanisms behind climate-induced changes in infectious disease risk and illustrate how a quantitative, ecophysiological framework can predict the response of different host-pathogen relations to climate warm-

ing. We mainly focus on changes in temperature, which have been more thoroughly explored both empirically and theoretically, relative to other environmental variables. We consider impacts of climate change on human diseases and on pathogens affecting species of conservation or economic concern, including agroecosystems [box S3 (8)]. A crucial need remains for long-term ecological studies that examine the consequences of climate-disease interactions for entire communities and ecosystems, as well as for efforts that couple effective disease forecasting models with mitigation and solutions.

Ecophysiology of Host-Pathogen Interactions

More than a century of research has firmly established that temperature and other climatic variables strongly affect the physiology and demography of free-living and parasitic species [e.g., (15)], with effects on behavior, development, fecundity, and mortality (16). Because these effects can be nonlinear and sometimes conflicting, such as warmer temperatures accelerating invertebrate development but reducing life span, a central challenge has been to identify the net outcomes for fitness (1). For infectious diseases, this challenge is compounded by the interactions between at least two species—a host and a pathogen—and often vectors or intermediate hosts, which make the cumulative influence of climate on disease outcomes elusive [e.g., (17, 18)].

Immune defenses are physiological processes crucial for predicting changes in disease dynamics. Warmer temperatures can increase immune enzyme activity and bacterial resistance for insects, such as the cricket *Gryllus texensis* (19). Positive effects of temperature on parasite growth and replication, however, might outweigh greater immune function of the host. In gorgonian corals, for example, warmer temperatures increase cellular and humoral defenses (20), but because coral pathogens also replicate faster under these conditions, disease outbreaks have coincided with warmer sea temperatures in the Caribbean (Fig. 1) (4, 5). Warm temperatures also can lower host immunity; for example, melanization and phagocytic cell activity in mosquitoes are depressed at higher temperatures (21). In addition, increased climate variability can interfere with host immunity, as illustrated by decreased frog resistance to the chytrid fungus *Batrachochytrium dendrobatidis* (Bd) in response to temperature fluctuations (14). Even though Bd grows best in culture at cooler temperatures, which suggests that warming should reduce disease, incorporating variability-induced changes in host resistance suggests a more complex relation between climate change and Bd-induced amphibian declines (22). These issues are important for predicting the immunological efficiency of ectotherms outside of their typical climate envelope.

One promising approach for predicting how host-pathogen interactions respond to climate

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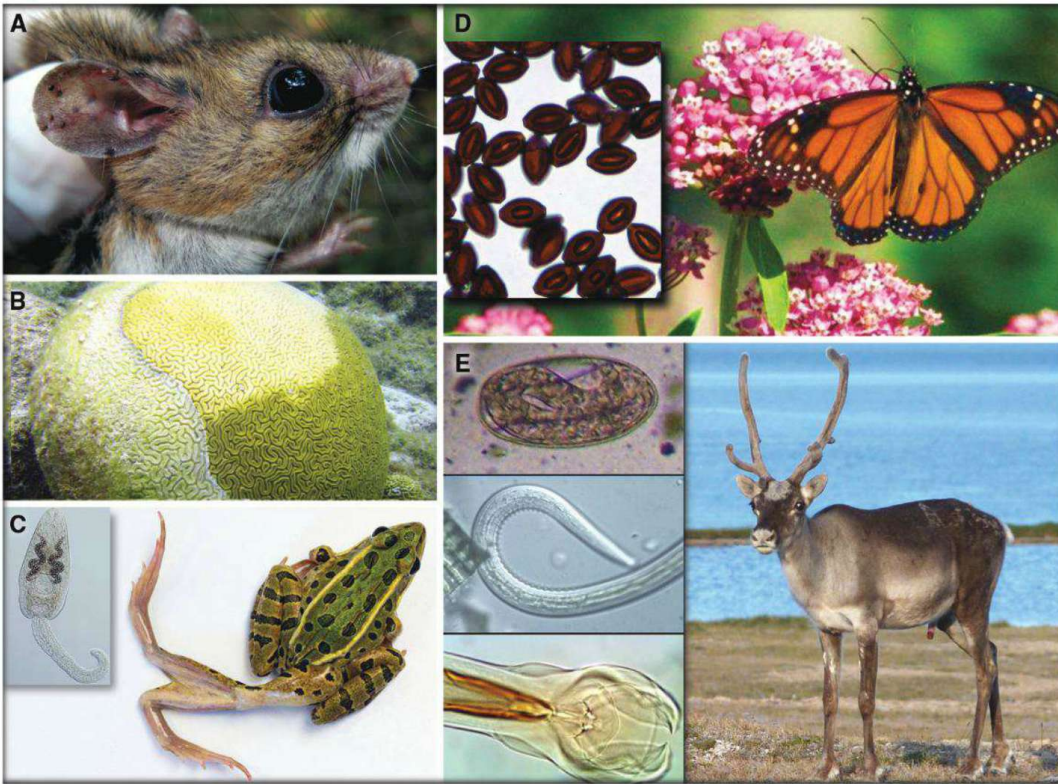


Fig. 1. Animal-parasite interactions for which field or experimental studies have linked climate change to altered disease risk. (A) Black-legged ticks, *Ixodes scapularis*, vectors of Lyme disease, attached to the ears of a white-footed mouse, *Peromyscus leucopus*, show greater synchrony in larval and nymphal feeding in response to milder climates, leading to more rapid Lyme transmission. (B) Caribbean coral (*Diploria labyrinthiformis*) was affected by loss of symbionts, white plague disease, and ciliate infection during the 2010 warm thermal anomaly in Curaçao. (C) Malformed leopard frog (*Lithobates pipiens*) as a result of infection by the cercarial stage (inset) of the multihost trematode *R. ondatrae*; warming causes nonlinear changes in both host and parasite that lead to marked shifts in the timing of interactions. (D) Infection of monarchs (*D. plexippus*) by the protozoan *O. elektrosirrha* (inset) increases in parts of the United States where monarchs breed year-round as a result of the establishment of exotic milkweed species and milder winter climates. (E) Infection risk with *O. gruehneri* (inset shows eggs and larvae) the common abomasal nematode of caribou and reindeer (*R. tarandus*), may be reduced during the hottest part of the Arctic summer as a result of warming, which leads to two annual transmission peaks rather than one (e.g., Fig. 3C). Photo credits (A to E): J. Brunner, E. Weil, D. Herasimtschuk, S. Altizer, P. Davis, S. Kutz.

warming involves infusing epidemiological models with relations derived from the metabolic theory of ecology (MTE). This approach circumvents the need for detailed species-specific development and survival parameters by using established relations between metabolism, ambient temperature, and body size to predict responses to climate warming (23). One breakthrough study (12) used MTE coupled with traditional host-parasite transmission models to examine how changes in seasonal and annual temperature affected the basic reproduction number (R_0) of stronglyid nematodes with direct life cycles and transmission stages that are shed into the environment. By casting R_0 in terms of temperature-induced tradeoffs between parasite development and mortality, this approach facilitated both general predictions about how infection patterns change with warming and, when parameterized for *Ostertagia gruehneri*, a nematode of caribou and reindeer (Fig. 1), specific projections that corresponded with the observed temperature dependence of parasite stages. More-

over, this model predicted a shift from one to two peaks in nematode transmission each year under warming conditions (Fig. 3C), a result consistent with field observations (12, 24).

In some cases, ecophysiological approaches must consider multiple host species and parasite developmental stages that could show differential sensitivity to warming. Such differential responses can complicate prediction of net effects, especially for ectothermic hosts with more pronounced responses to temperature. For instance, because both infectivity of a trematode parasite (*Ribeiroia ondatrae*) and defenses of an amphibian host (*Pseudacris regilla*) increase with temperature, maximal pathology (limb malformations) (Fig. 1) occurs at intermediate temperatures (25). Other work showed that the virulence of both a coral fungus (*Aspergillus sydowii*) and protozoan (*Aplanochytrium* sp.) increased with temperature, probably because pathogen development rate continued to increase in a temperature range where coral defenses were less potent (26). Thus, the

ideal approach will be an iterative one that combines metabolic and epidemiological modeling to predict general responses and to identify knowledge gaps, followed by application of models to specific host-pathogen interactions.

Community Ecology, Biodiversity, and Climate Change

Host-pathogen interactions are embedded in diverse communities, with climate change likely leading to the loss of some host-pathogen interactions and the gain of novel species pairings. In some cases, pathogen extinction and the loss of endemic parasites could follow from climate change, potentially reducing disease or conversely releasing more pathogenic organisms from competition. In other cases, multiple pathogens can put entire host communities at risk of extinction. Although ecosystems of low biodiversity, such as occur in polar regions, can be particularly sensitive to emerging parasitic diseases (27), most knowledge of community-wide responses stems from tropical marine systems. For example, the wider Caribbean region is a “disease hot spot” characterized by the rapid, warming-induced emergence of multiple new pathogens that have caused precipitous coral declines with ecosystem-wide repercussions (28, 29). Impacts of climate-induced changes in disease can be especially large

when the host is a dominant or keystone species. For example, near extinction of the once-dominant, herbivorous abalone (genus *Haliotis*) by warming-driven rickettsial disease caused pervasive community shifts across multiple trophic levels (5). Similarly, seagrass (*Zostera marina*) declines caused by infection with the protist *Labyrinthula zosterae*, which correlates positively with warming, have degraded nursery habitats for fish and migratory waterfowl and caused the extinction of the eelgrass limpet (30).

Microbial communities, which are often part of the extended phenotype of host defenses, are also likely to respond to climate changes. For instance, warming sea-surface temperatures in coral reefs can inhibit the growth of antibiotic-producing bacteria, sometimes causing microbial communities to shift from mutualistic to pathogenic (31). In agroecosystems, higher temperatures can suppress entomopathogenic fungi and antibiotic production by bacterial mutualists in plants (32). Warming also underlies bacterial shifts from endosymbiotic

to lytic within host amoebas that live in human nasal passages, increasing the potential risk of respiratory disease (33). Thus, effects of warmer temperatures on the diversity and function of commensal or mutualist microbes could promote pathogen growth and pest outbreaks.

From a broader perspective, biodiversity loss is a well-established consequence of climate change (16, 34) and can have its own impact on infectious diseases. For many diseases of humans, wildlife, and plants, biodiversity loss at local or regional scales can increase rates of pathogen transmission (35–37). This pattern can result from several mechanisms, including the loss of the dilution effect (36). For example, lower parasite diversity could allow more pathogenic species to proliferate when endemic and competing parasites are lost from a system (36). Climate warming can also weaken biotic regulation of disease vectors by inhibiting their predators (38) and competitors (39). Interactions between biodiversity and infectious disease underscore the need to put climate-disease interactions into the broader context of other forms of global change, such as land-use change and habitat loss, when extending predictions from focused host-pathogen interactions to larger spatial and taxonomic scales.

Shifts in Behavior, Movement, and Phenology of Hosts and Parasites

Changes in climate are already affecting the phenology of interactions between plants and pollinators, predators and prey, and plants and herbivores (16). Climate-induced shifts in phenology and species movements (40) will likely affect disease dynamics. Many species are already moving toward higher elevations or latitudes (41), and an open question is whether these shifts could disrupt established interactions or bring novel groups of hosts and pathogens into contact (42). For instance, the range expansion of the Asian tiger mosquito (*Aedes albopictus*) across Europe and the Americas has created the potential for novel viral diseases such as Chikungunya to invade (10); this pathogen is already expanding in geographic range, and a recent outbreak in Europe emphasizes the need for surveillance and preparedness. Along eastern North America, warming sea temperatures and changes in host resistance facilitated a northward shift of two oyster diseases into previously unexposed populations (5).

Migratory species in particular can be sensitive to climate change (41), with the routes and timing of some species' migrations already shifting with climate warming (16). Long-distance migrations can lower parasite transmission by allowing hosts to escape pathogens that accumulate in the environment or by strenuous journeys that cull sick animals (43). In some cases, milder winters can allow previously migratory host populations to persist year-round in temperate regions (44); this residency fosters the build-up of environmental transmission stages, and mild

winters further enhance parasite over-winter survival (2). A case study of monarch butterflies (*Danaus plexippus*) and the protozoan parasite *Ophryocystis elektroscirrha* (Fig. 1) provides support for climate-warming shifts in migration and disease. Monarchs typically leave their northern breeding grounds in early fall and fly to Mexican wintering sites. Milder winters, combined with increased planting of exotic host plants, now allow monarch populations to breed year-round in parts of the United States (45). Relative to migratory monarchs, winter-breeding monarchs suffer from higher rates of infection (43). Similarly, migration is considered an important parasite avoidance strategy for barren-ground caribou (24), but the loss of sea ice with climate warming will likely inhibit migrations and prevent them from seasonally escaping parasites (46). Thus, diminishing migration behaviors among animals that use seasonal habitats can increase the transmission of infectious diseases.

Changes in the timing of vector life stages and feeding behavior can also arise from interactions between climate and photoperiod. For several tick-borne infections (Fig. 1), pathogens are sequentially transmitted from infected vertebrate hosts to naïve larval tick vectors, and from infected nymphal ticks to naïve vertebrate hosts. Asynchrony in the seasonal activity of larval and nymphal ticks can delay transmission and select for less virulent strains of the Lyme bacterium *Borrelia burgdorferi* (47), whereas synchrony allows for more rapid trans-

mission and the persistence of virulent strains. In the case of tick-borne encephalitis (TBE), viral transmission occurs directly between co-feeding ticks; thus, viral maintenance requires synchronous larval and nymphal feeding (48). Because synchrony of larval and nymphal ticks characterizes milder winter climates, climate change could increase tick synchrony and the transmission and virulence of several tick-borne infections.

Changes in the timing of shedding or development of environmental transmission stages could result from climate warming. Some parasites could experience earlier hatching, exposure to hosts earlier in the season, and encounters with earlier (and often more sensitive) life stages of hosts. For example, a long-term data set of lake plankton showed that warming shifted fungal prevalence patterns in diatom hosts from acute epidemics to chronic persistence, in part because of faster transmission and more widespread host population suppression under warmer temperatures (49). In contrast, Brown and Rohani (50) argued for the opposite outcome with respect to avian influenza (AI) in reservoir bird hosts. Climate-driven mismatch in the timing of bird migration and their prey resources (e.g., horseshoe crab eggs) amplified variability in epidemiological outcomes: Although mismatch increased the likelihood of AI extinction, infection prevalence and spillover potential both increased in cases where the virus persisted.

Plasticity in parasite traits could allow parasites with environmental transmission stages to

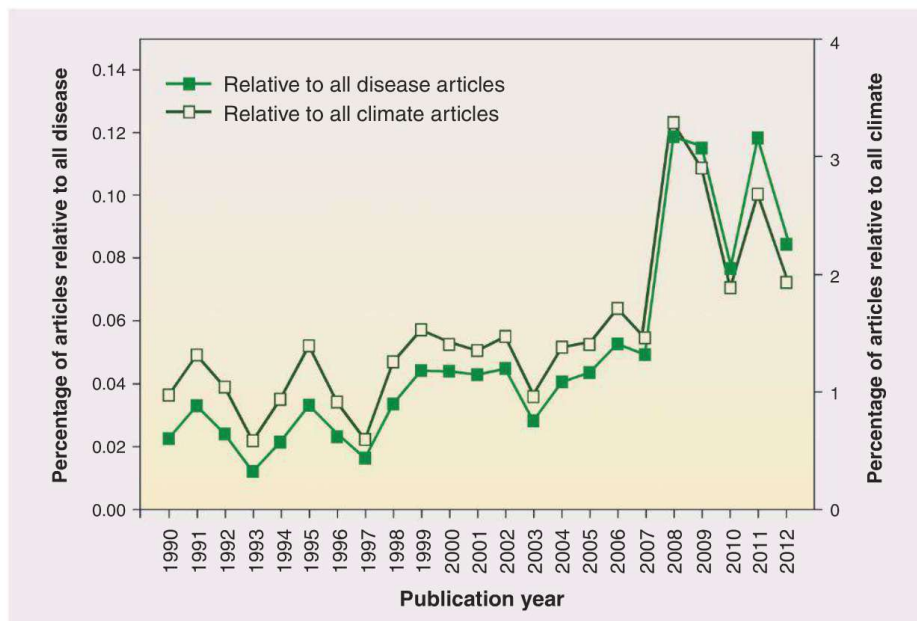


Fig. 2. Rising interest in climate-disease interactions. Research focused on associations between infectious disease and climate change has increased steadily over the past 20 years. After correcting for total research interest in climate change (open symbols) or infectious disease (closed symbols), the frequency of papers referencing a climate-disease link in the title has nearly doubled over this period, based on long-term publication trends following a Web of Science search of article titles (1990 to 2012). Search criteria and statistical analyses are provided in the supplementary materials, and the total number of climate change–infectious disease papers identified by our search criteria ranged from 5 to 117 publications per year.

respond more rapidly to climate warming. For example, arrested development (hypobiosis) of the nematode *O. gruehneri* within its caribou

host is a plastic trait more commonly expressed in areas with harsher winters as compared with milder climates (51). This arrested state prevents

wasted reproductive effort for the parasites, because eggs produced in late summer in colder regions are unlikely to develop to infective-stage larvae by fall. Ultimately, plasticity in life history traits could speed parasite responses to changing environments and allow parasites to deal with climate instabilities (e.g., a series of severe winters interspersed by mild), relative to the case where selection must act on genetically variable traits (52). For example, if climate warming extends the transmission season for *O. gruehneri* on tundra, a rapid decrease in the frequency of nematode hypobiosis could shorten the life cycle and increase infection rates.

Consequences for Conservation and Human Health

Climate change is already contributing to species extinctions, both directly and through interactions with infectious disease (53). Roughly one-third of all coral species and the sustainability of coral reef ecosystems are threatened by human activities, including climate warming and infectious diseases (5). In contrast to tropical marine systems, the Arctic is a less diverse and minimally redundant system that is warming at least twice as fast as the global average (54) and simultaneously experiencing drastic landscape changes from an expanding human footprint. Altered transmission dynamics of parasites, poleward range expansion of hosts and parasites, and disease emergence coincident with climate warming or extremes have all been reported in the Arctic (27, 55). Together, these phenomena are altering host-parasite dynamics and causing endemic Arctic species—unable to compete or adapt rapidly enough—to decline (56). Changes in wildlife health can also compromise the livelihoods and health of indigenous people who depend on wildlife for food and cultural activities (57).

In humans, exposure to diarrheal diseases has been linked to warmer temperatures and heavy rainfall (58). Human infections of cholera, typically acquired through ingestion of contaminated water (in developing countries) or undercooked seafood (in the developed world), affect millions of people annually with a high case-fatality rate. Coastal *Vibrio* infections are associated with zooplankton blooms, warmer water, and severe storms (3). For example, in the Baltic Sea, long-term warming and temperature anomalies have been linked to increased disease from *Vibrio vulnificus*, which was first reported in 1994 along the German coast after an unusually warm summer (3). Long-term sea surface warming can increase the geographic range, concentration, and seasonal duration of *Vibrio* infections, as seen in coastal Chile, Israel, and the U.S. Pacific Northwest. Modeling approaches indicate that *Vibrio* illnesses from the Baltic region could increase nearly twofold for every 1°C increase in annual maximum water temperature (3).

Human mosquito-borne diseases, such as malaria and dengue fever, are frequently proposed

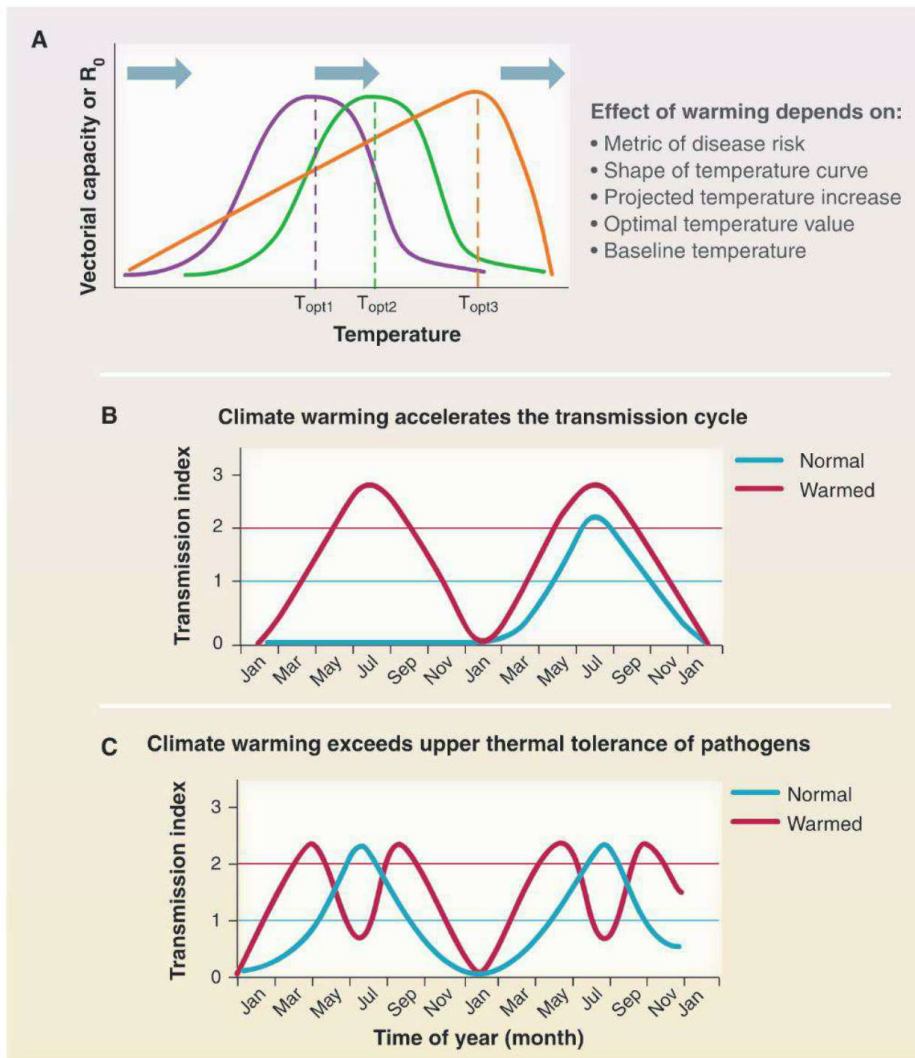


Fig. 3. Theoretical underpinnings and categorization of disease responses to climate change. Pathogen responses to climate change depend on thermal tolerance relative to current and projected conditions across an annual cycle. **(A)** Gaussian curves relating temperature to a metric of disease risk suggest symmetrical temperature zones over which warming will increase and decrease transmission, whereas left-skewing [a common response for many terrestrial ectotherms, including arthropod vectors (74)] indicates greater potential for pathogen transmission to increase with warming [box S2 (8)]. Bold arrows represent geographic gradients that span cool, warm, and hot mean temperatures, which indicate that the net effect of warming (at point of arrows) depends on whether temperatures grow to exceed the optimum temperature (T_{opt}) for disease transmission. Projected changes in disease will further depend on the starting temperature relative to T_{opt} , the magnitude of warming, measurement error, adaptation, and acclimation. **(B)** Pathogens at their northern or altitudinal limits might show range expansion and nonlinear shifts in their life cycle in response to warmer temperatures (red) relative to baseline (blue). For example, a shift from 2- to 1-year cycles of transmission has occurred for the muskox lungworm (27). This outcome could generate sporadic disease emergence in a naïve population (if extremes in temperature allow only occasional invasion and/or establishment), or could gradually increase prevalence and establishment. **(C)** At the low-latitude or low-altitude extent of a pathogen's range, where temperature increases could exceed the pathogen's thermal optimum, transmission might be reduced, or we might see the emergence of a bimodal pattern whereby R_0 peaks both early and late in the season, but decreases during the midsummer [as in the case of the arctic *O. gruehneri*–reindeer example (12)]. In **(B)** and **(C)**, the lower blue line represents $R_0 = 1$, above which the pathogen can increase; values above the pink line represent severe disease problems owing to a higher peak of R_0 and a greater duration of time during which $R_0 > 1$.

as cases where vector and disease expansion into the temperate zone could follow from climate warming (59). However, some researchers have argued that ranges will shift with warming, rather than expand, and that the best predictors of infection risk are economic and social factors, especially poverty (17, 60). Controversy has also arisen over which climatic variables are most important in delimiting the distributions of these diseases [boxes S1 and S2 (8)]. Detecting impacts of climate change on human vector-borne diseases remains difficult, in part, because active mitigations, such as vector-control, antimicrobials, and improved infrastructure can complicate detection of a climate signal. Several unresolved issues include identifying conditions under which climate warming will cause range expansions versus contractions, understanding the impact of increasing variability in precipitation, and determining the additional economic costs associated with increased disease risk caused by warming.

Ultimately, the societal implications of climate-driven shifts in diseases of humans, crops, and natural systems will demand solutions and mitigation, including early-warning programs. Recently, a forecasting system linking global coupled ocean-atmosphere climate models to malaria risk in Botswana allowed anomalously high risk to be predicted and anticipatory mitigations to be initiated (61). Forecasting is well-established in crop disease management and leads to improved timing of pesticide application and deployment of planting strategies to lower disease risk [box S3 (8)]. Modeling efforts to better predict crop loss events are also tied to improved insurance returns against losses (2). Similarly, accurate forecasting programs for coral bleaching have become a mainstay of marine climate resilience programs (62) and are leading to the development of coral disease-forecasting algorithms (63). Appropriate management actions under outbreak conditions include reef closures to reduce stress and transmission, culling of diseased parts of some colonies, and increased surveillance (64). In the ocean, efforts are also under way to increase the resilience of marine ecosystems to disease, including developing no-fishing zones and reducing land-based pollution that can introduce new pathogens (5).

Outlook and Future Challenges

Climate change will continue to limit the transmission of some pathogens and create opportunities for others. To improve predictions and responses we need to deepen our understanding of mechanistic factors. Although the initial climatic drivers to be explored should be temperature variables (both mean and variability), because the data are available and we understand the mechanisms at work, future work must concurrently explore the effects of precipitation, relative humidity, and extreme events. In particular, models are needed that combine the principles of ecophysiology and MTE (23) with epidemiological response variables, such

as R_0 or outbreak size, and that are designed to accommodate distinct pathogen types (e.g., vector-borne, directly transmitted, or complex life cycle) and host types (ectotherm versus endotherm) (12). These models should be applied, by using climate-change projections, to evaluate how broad classes of pathogens might respond to climate change. Building from this foundation, the next step is to extend such general models to specific pathogens of concern for human health, food supply, or wildlife conservation, which will require empirical parameterization, with attention to the on-the-ground conditions. Modeling efforts should be integrated with experiments to test model predictions under realistic conditions, and with retrospective studies to detect the “fingerprint” of climate-induced changes in infection.

Scientists still know relatively little about the conditions under which evolution will shape host and pathogen responses to climate change. Although evolutionary change in response to climate warming has been reported for some free-living animals and plants, the evidence remains limited (52). Even less is known about how climate change will drive host-pathogen evolution. Corals have multiple levels of adaptation to intense selection by thermal stress that could also affect resistance to pathogens, including symbiont shuffling of both algae and bacteria, and natural selection against thermally intolerant individuals (65). In oysters (*Crassostrea virginica*), warming might have contributed to increased resistance to the protozoan multinucleated sphere X (MSX) disease (66), but climate variability might also slow the evolution of oyster resistance (67). In cases where increased rates of transmission follow from warming, selection could favor higher pathogen virulence, although examples are now unknown.

A persistent challenge involves the ability to detect changes in disease risk with climate across different systems. In the oceans, for example, impacts of disease on sessile hosts like corals, abalones, and oysters are readily apparent, and for terrestrial systems, clear impacts are seen for plant diseases and some wildlife-helminth interactions. But for highly mobile species and many human diseases, detecting signals of climate change remains problematic. For these less tractable systems, long-term ecological studies that examine the past distributions of pathogens, important hosts, and severity of diseases are indispensable. Permanent repositories of intact physical specimens, as well as their DNA, can provide records of diversity that will be critical resources as new methodologies become available (68, 69). Moreover, new technologies can detect variability in physiological processes and gene expression and can improve climate projections from global circulation models. Sophisticated experimental designs conducted under appropriate ranges of environmental conditions and retrospective studies to identify past climatic effects on disease (5, 70) will help advance predictive power.

An additional key challenge is predicting the impacts of climate-disease interactions for human societies and gauging how these compare with other components of climate change, such as the loss of arable land. By affecting food yields and nutrition, water quality and quantity, social disorder, population displacement, and conflict, past climate changes have long influenced the burden of infectious disease in many human societies (71, 72). Predicting the regions where humans and natural systems are most vulnerable to pressures from infectious disease and how these pressures will translate to changes in global health and security constitute critical research priorities (73). Building a mechanistic understanding of climate-disease interactions will allow public health interventions to be proactive and will facilitate effective responses to new or expanding health threats. Surveillance programs capable of detecting pathogen or disease emergence are essential and, in many instances, predicting and detecting local-scale impacts might be more important than predicting global-scale changes. To this end, the value of engaging local communities in disease surveillance is increasingly recognized, with the goal of advancing science on climate-disease linkages for practical solutions to protecting human and wildlife health.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/341/6145/514/DC1
Materials and Methods
Supplementary Text
Fig. S1
Boxes S2 to S3
References (75–95)

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REVIEW

Ecological Consequences of Sea-Ice Decline

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After a decade with nine of the lowest arctic sea-ice minima on record, including the historically low minimum in 2012, we synthesize recent developments in the study of ecological responses to sea-ice decline. Sea-ice loss emerges as an important driver of marine and terrestrial ecological dynamics, influencing productivity, species interactions, population mixing, gene flow, and pathogen and disease transmission. Major challenges in the near future include assigning clearer attribution to sea ice as a primary driver of such dynamics, especially in terrestrial systems, and addressing pressures arising from human use of arctic coastal and near-shore areas as sea ice diminishes.

As one of Earth's major biomes, sea ice not only comprises unique ecosystems in, on, and under the ice itself but also strongly influences patterns and processes in adjacent terrestrial ecosystems (1, 2) (Fig. 1). Sea ice harbors an array of microorganisms, provides critical habitat for vertebrates, and influences terrestrial productivity and diversity in the Arctic, where 80% of low-lying tundra lies within 100 km of seasonally ice-covered ocean (3–5). Ice-loss-driven amplification of arctic warming is a potentially important driver of ecological dynamics in the region, where seasonal temperature limitation is an important constraint on productivity (6). Here, we synthesize recent developments in the study of ecological

responses to arctic sea-ice decline and highlight the importance of sea-ice loss as a driver of ecological dynamics in both marine and terrestrial systems.

Record of Recent Sea-Ice Loss

One of the most conspicuous consequences of recent anthropogenic warming has been declining annual minimum extent of arctic sea ice (7). Over the past several decades, the Arctic has warmed at twice the global rate, with sea-ice loss accelerating (8) (Fig. 2A), especially along the coasts of Russia, Alaska, and the Canadian Archipelago (Fig. 2B). The sea ice's annual minimum reached a record low in 2012. Arctic sea-ice loss has exceeded most model pro-

jections (9) and is unprecedented in the past 1.5 millennia (10).

Sea-ice loss is most commonly discussed as an indicator of arctic warming (11), but it is also a major factor in amplification of warming in the Arctic through feedback deriving from declining surface albedo (6). In 2007, the year of second-lowest arctic sea-ice extent on record, sea ice loss accounted for a large portion of warming over land north of 60° (12). Further, much of arctic near-surface warming over the past three decades is attributable to declining sea ice concentration (13), and land-surface warming is linked to summer sea-ice loss in global climate models (14).

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Direct Effects of Sea-Ice Loss

Primary producers dependent upon sea ice as their habitat underpin the entire marine food web of the Arctic (Fig. 1A). The loss of over 2 million km² of arctic sea ice since the end of the last century (Fig. 2A) (10) represents a stunning loss of habitat for sea-ice algae and sub-ice phytoplankton, which together account for 57% of the total annual primary production in the Arctic Ocean (15). The seasonal timing of the ice algae bloom, driven by light penetration through thinning sea ice, is critical to the successful reproduction of zooplanktonic copepod grazers, and the timing of the subsequent phytoplankton bloom as the ice edge retreats is critical to the growth and survival of copepod offspring (15). These two annual pulses of productivity, including the release of organic material from seasonally melting ice, fuel the arctic marine food web (2).

Disruption of the seasonality of the ice algal and phytoplankton blooms by ice thinning, accelerated melt timing, and an increase in the length of the annual melt season by 20 days over the past three decades (16) has created mismatches for the timing of zooplankton production, with consequences for higher consumers (17, 18). Earlier seasonal sea-ice melt and earlier phytoplankton blooms may shorten the length of the annual window of arctic marine primary productivity (19), affecting zooplankton production and that of the arctic cod that feed on them (20) as well as their seabird and marine mammalian predators (2, 21) (Fig. 1B).

Warming-related reductions in sea-ice thickness and snow cover on sea ice in the Arctic Ocean have also been associated with increased sub-ice primary production. A midsummer phytoplankton bloom below the sea ice in 2011 was attributed to enhanced light transmission through a thin layer of first-year ice (22). Hence, replacement of thick, multiyear ice by thin, first-year ice as the Arctic warms may contribute to increases in the frequency and magnitude of algae and phytoplankton blooms. However, the roles of sea-ice loss and ocean freshening in the tradeoffs between light versus nutrient limitation of arctic marine primary productivity remain poorly understood (1). Freshening of the euphotic layer associated with sea-ice melt may ultimately reduce nutrient availability for phytoplankton, limiting their productivity despite increased solar input with sea-ice retreat (23). Also, increased solar irradiance of sea-ice algae through thinning ice reduces their fatty acid content and quality as forage for marine copepod grazers (24). Furthermore, freshening of the Arctic Ocean due to increased meltwater from sea ice and runoff from coastal rivers is associated with the replacement of larger nanoplankton by smaller picoplankton, reducing the efficiency of seasonal energy transfer in marine food webs (25).

Vertebrate species dependent upon sea ice for foraging, reproduction, and resting are also directly affected by sea-ice loss and thinning (3). Examples

of marine vertebrates adversely affected by sea-ice decline and longer ice-free seasons include declines in body condition and abundance of polar bears (26) and loss of critical habitat for reproduction and offspring provisioning by ringed seals (27). Pacific walrus have recently displayed greater use of shoreline haul-out areas and declining abundance in portions of their range, as retreating near-coastal sea ice has reduced their access to critical shallow water foraging from the ice edge (28). Mass

mortality among Pacific walrus along the coast of the Chukchi Sea in Alaska has been attributed to loss of sea ice over the continental shelf (29).

Indirect Effects of Sea-Ice Loss

Sea-ice loss may also influence ecological dynamics indirectly through effects on movement, population mixing, and pathogen transmission. For populations and species currently isolated only during the summer ice-free season in the

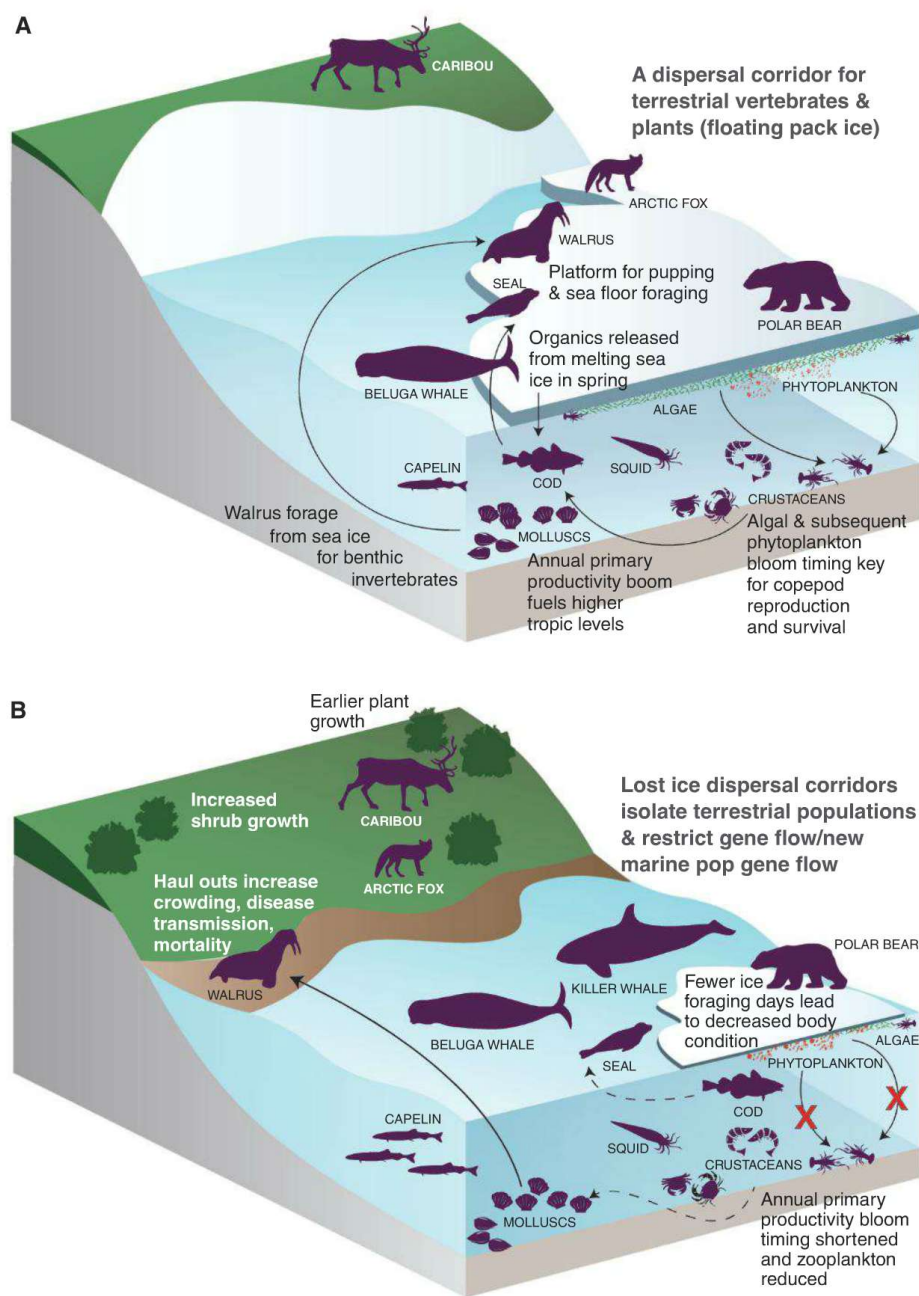


Fig. 1. Ecological interactions influenced by sea ice. The sea-ice biome influences the abundance, distribution, seasonality, and interactions of marine and terrestrial species by its presence (A). It is unique for its complete seasonal disappearance in portions of its distribution. Lengthening of this annual period of absence and an overall decline in ice extent, thickness, and stability will have considerable consequences for these species and interactions (B).

Arctic, declining annual presence of sea ice will reduce trans-ice and interisland migrations outside of the summer season. Sea-ice loss and a lengthening of the ice-free season will thus increase genetic isolation among populations of such species. Sea ice is the strongest predictor of genetic differentiation among arctic fox populations (30). In the Canadian Arctic Archipelago, interisland and island-mainland migration can promote genetic rescue of isolated wolf populations (31). The loss of sea ice that seasonally connects these populations will render such genetic rescue increasingly unlikely.

In species for which sea ice acts as a barrier to dispersal, its loss and a lengthening of the ice-free season will increase population mixing, reducing genetic differentiation. Perennial sea ice likely maintains genetic divergence between North Pacific and North Atlantic populations of walrus (32) and some whales (33). Loss of sea ice will also increase contact among closely related species for which it currently acts as a barrier to mixing, in-

creasing the likelihood of hybridization. For instance, at least seven pairs of arctic and subarctic marine mammals hybridize, and many more hybridizations are expected with sea-ice loss (34). Observed hybridization between polar bears and grizzly bears may be the result of increasing inland presence of polar bears as a result of prolonged ice-free seasons (34). Loss of sea ice may reduce arctic faunal diversity if it promotes hybridization among populations, species, and genera currently isolated by ice (34).

Arctic warming and sea-ice loss will also facilitate invasions by new hosts, pathogens, and disease vectors. The projected decrease in sea-ice cover in arctic Canada will increase contact between eastern and western arctic species, promoting mixing of pathogen communities previously isolated. Phocine distemper virus, currently endemic to pinnipeds of the eastern Arctic, may spill over to western arctic species where it is currently absent. Mixing of Atlantic and Pacific pathogen communities that have been ecolog-

ically and evolutionarily isolated may be expected across a range of marine species, with important implications for the health of populations previously not exposed to them. For walrus, reduced sea-ice cover forces increased use of shoreline haul-outs (Fig. 1B), increasing the local density of animals. This promotes transmission of environmentally and density-dependent pathogens. Additionally, increased time spent on land by marine species may enhance transmission of pathogens between them and terrestrial species (35).

Changes in animal behavior as a result of sea-ice loss may also alter patterns of pathogen exposure. In the Canadian Arctic, later freeze-ups and increased shipping traffic could shift or prevent the annual migration of the Dolphin and Union caribou herd. Because migration poses benefits for reducing parasitism, such a change may increase parasite loads in this herd. Conversely, sea-ice loss may be beneficial in preventing pathogen introduction and disease epidemics to island ecosystems in cases where sea ice provides a corridor for pathogen transmission. Sporadic outbreaks of rabies on Svalbard are attributed to introduction by arctic foxes traversing sea ice from the Russian mainland (36). Reduction in sea ice would likely minimize or eliminate this movement.

Shifts in feeding ecology mediated by sea-ice loss may also alter the community of parasites within a host, particularly in the case of parasites with complex life cycles (37). For example, the diet of thick-billed murres in Hudson Bay has shifted from arctic cod to capelin (38), potentially affecting the occurrence of parasites transmitted through the food web. Similarly, sea-ice alteration of exposure of wildlife to environmental toxicants will have important impacts on the immune function of animal species and their ability to cope with existing and new pathogens (35).

Effects on Terrestrial Systems

Contributions of sea-ice loss to near-surface warming over land across the Arctic (13) indicate that earlier annual sea-ice melt and ice loss will influence seasonality in terrestrial systems. Local warming over land adjacent to areas of sea-ice loss is expected to increase terrestrial primary production for two reasons: Surface warming advances arctic soil thaw dates and delays soil freeze dates (39), and sea-ice loss is expected to promote permafrost warming up to 1500 km inland from the coastline (40).

In West Greenland, long-term monitoring of plant phenology at an inland site indicates a close association between the annual timing of the plant growing season and sea-ice extent (Fig. 3A) (41). Here, springs with low sea-ice extent are characterized by early green-up of vegetation. Advancement of the timing of the spring pulse of primary production, in turn, exacerbates trophic mismatch for caribou at the site (41), as it does for copepod grazers in the marine food web (17). At the same inland site, abundance of dwarf shrubs has

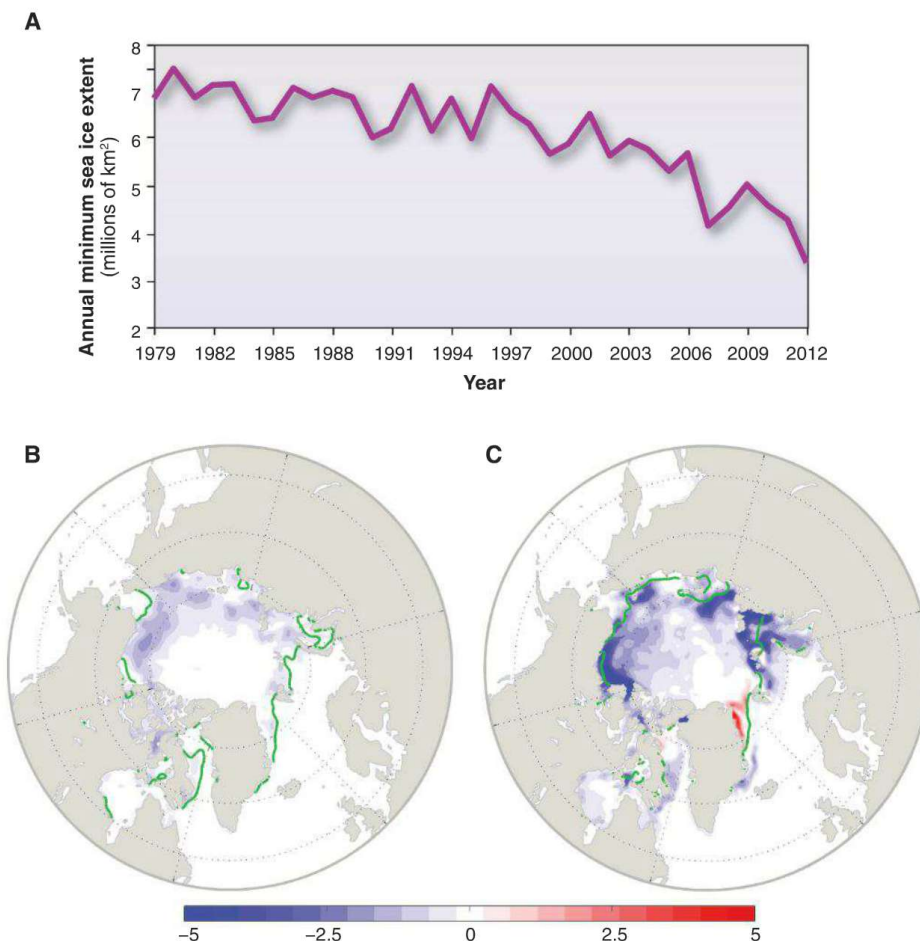


Fig. 2. Trends in arctic sea ice through time and space. Annual minimum sea-ice extent (A) has declined dramatically from 1979 to 2012. The percentage concentration loss per year in seasonal sea-ice minimum extent (July to September) has increased most between 1979 and 1999 (B) and between 2000 and 2011 (C) along the coasts of Russia, Alaska, and the Canadian Arctic Archipelago. The color bar indicates the direction of the sea-ice trend in percentage change per year; in the panels, the mean 15% concentration contour is shown in green. All data is from NASA Distributed Active Archive Center at the National Snow and Ice Data Center.

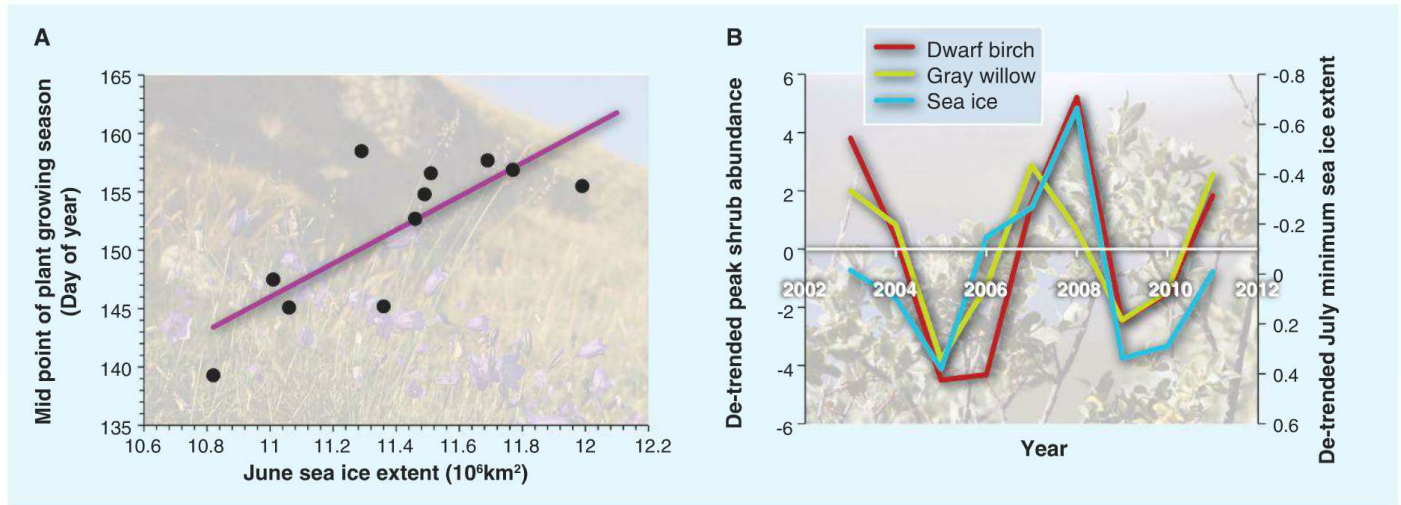


Fig. 3. Relations between sea ice and timing and abundance of terrestrial plant growth. (A) The annual midpoint of the plant growing season at an inland site in Greenland, when 50% of species have emerged on plots monitored between 1993 and 2011, is closely associated with Arctic-

wide sea-ice extent in June [data from (41)]. (B) Detrended annual peak aboveground abundance of dwarf shrubs [data from (42)], which have been increasing at the same site (42), displays a close association with July sea-ice extent in the previous year.

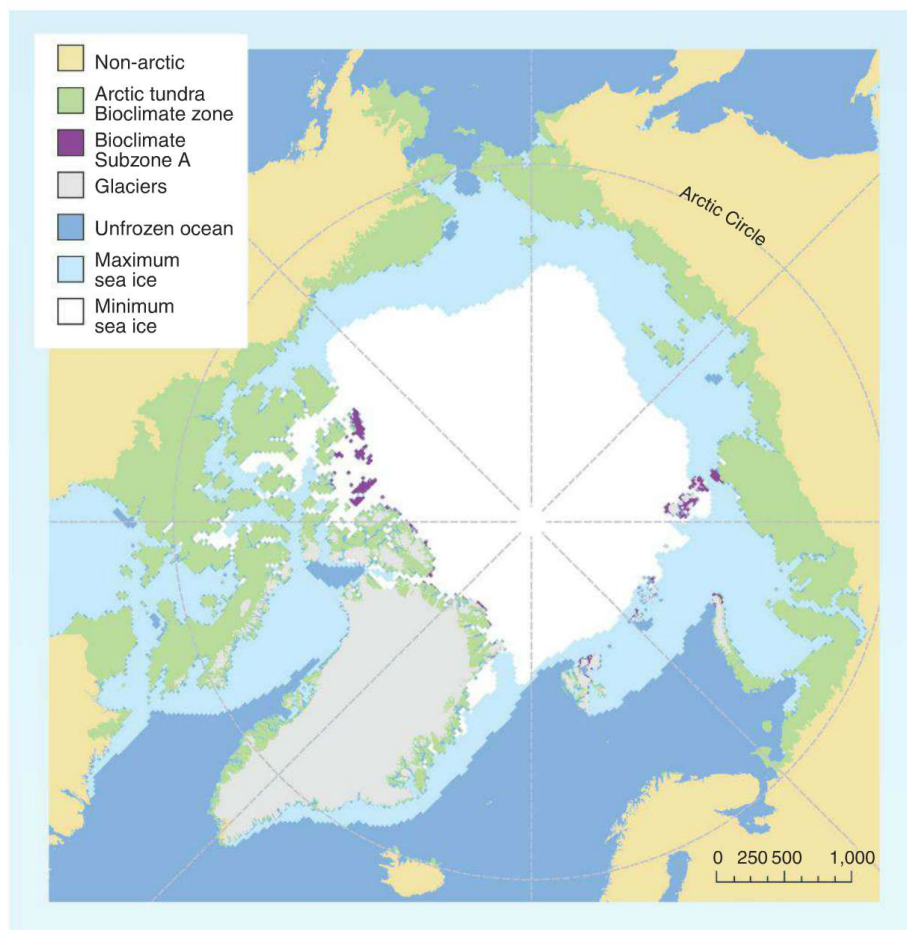


Fig. 4. Arctic terrestrial vegetation zones in relation to sea ice. The extent and locations of the arctic tundra bioclimate zone and bioclimate subzone A [boundaries of both from (44)] are closely related to the climatological maximum and minimum spatial extent of sea ice. The mean (1982 to 2010) maximum ice boundary (50% ice cover) is shown for week 22 (1 June), and the minimum ice

boundary (50% ice cover) is shown for week 35 (1 September). The tundra extent generally corresponds to the extensive presence of sea ice during the late winter and spring. Bioclimate subzone A relates to the presence of extensive ice cover during all of the summer and early autumn (45). Ice boundaries were determined from passive microwave data averaged for 1982 to 2012.

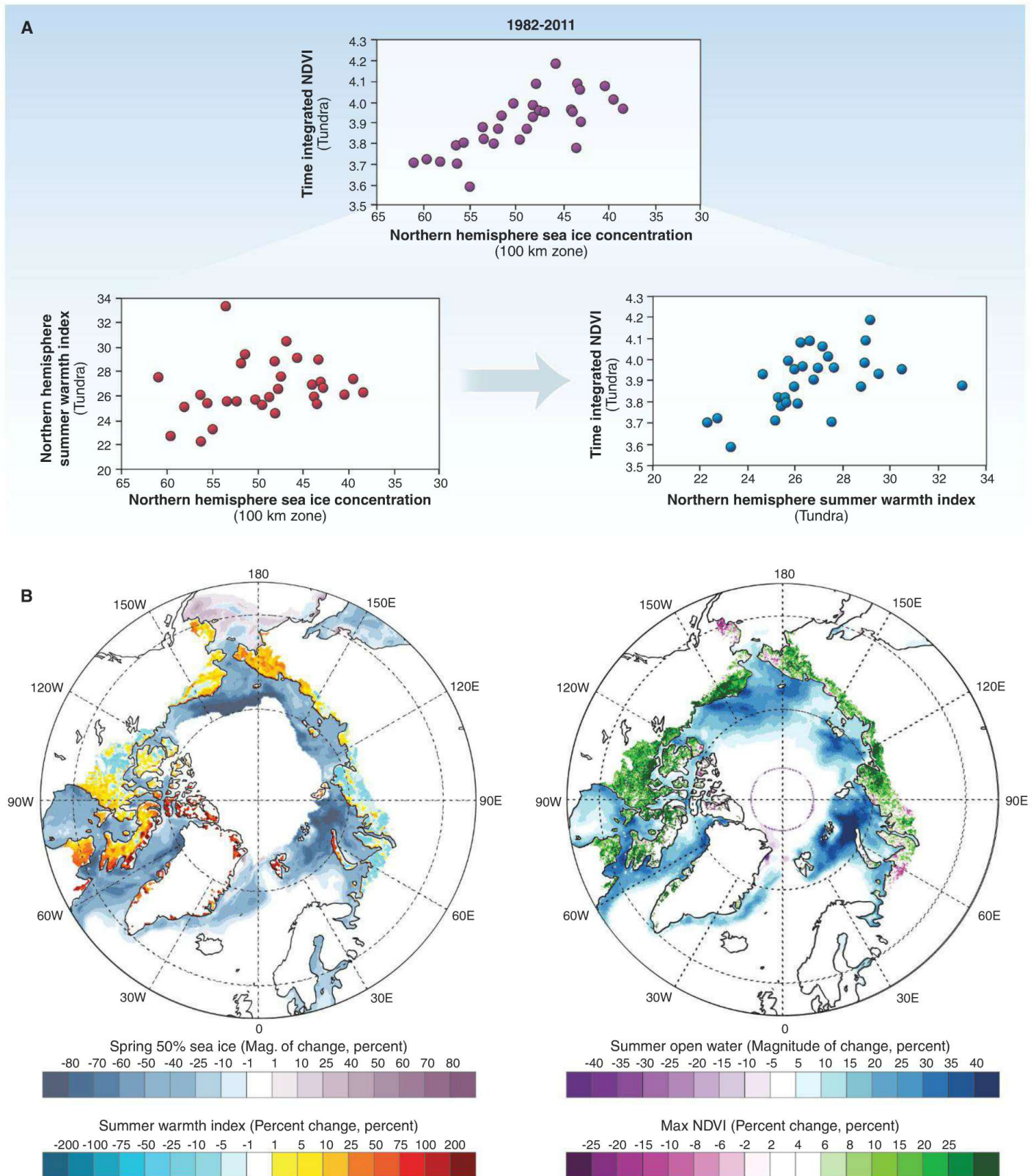


Fig. 5. Increasing arctic terrestrial primary production associated with sea-ice decline. (A) Coastal tundra primary productivity, shown as time-integrated NDVI, has increased in association with declining arctic sea-ice concentration or area (top). This is presumed to be driven by the relations between

sea-ice area and SWI (bottom left) and between SWI and NDVI (bottom right). **(B)** Pan-Arctic trends in SWI (left) and NDVI (right) [adapted from (4, 5)] vary spatially across the Arctic, but almost all locations experienced an increase in maximum NDVI and an increase in summer open water (right).



increased (42) and relates inversely to sea-ice extent during the previous growing season (Fig. 3B). Inferring causality between correlated time series is difficult but may be supported when the response displays a lagged relation to the presumed driver, as in this instance.

Increases in the abundance and cover of shrubs are occurring across the Arctic (43). In coastal and near-coastal areas, these increases are likely related to local warming driven by sea-ice loss. The entire arctic tundra biome is coupled with the marine system because of its extensive coastline (Fig. 4) and is especially vulnerable to sea-ice decline because of the strong climatic influence of the nearby sea ice. A unique area that will be particularly sensitive to sea-ice loss is bioclimate subzone A (Fig. 4) (44). Floristically depauperate and experiencing some of the largest and fastest temperature changes in the Arctic, this zone is likely to experience complete loss of summer sea ice in the next few decades, rendering it an endangered bioclimate subzone (45).

Associations between sea-ice decline and terrestrial primary productivity are also evident at larger scales across the Arctic. Biome-scale evidence for a relationship between sea-ice decline and increases in terrestrial primary productivity derives mainly from satellite data. Between 1982 and 2011, as near-coastal sea-ice area declined, the summer warmth index (SWI) for low-elevation tundra along the Arctic Ocean increased, precipitating an increase in vegetation production captured by Normalized Vegetation Difference Index (NDVI) data (4, 5) (Fig. 5A). The relationship between SWI and sea-ice extent is largely negative for the entire Northern Hemisphere, indicating warming associated with sea-ice loss, but varies among regions such as Eurasia and North America (fig. S1). Moreover, NDVI trends and relations to sea-ice extent vary across the Arctic (46) (Fig. 5B), suggesting that other factors likely interact with abiotic drivers associated with sea-ice loss to influence variation in terrestrial primary productivity across the tundra biome.

Increases in terrestrial primary productivity related to sea-ice decline and the consequent increase in land surface temperatures have the potential to alter ecosystem carbon flux (47). Modeling of measurements of CO₂ flux from West Greenland indicates a doubling of carbon uptake concordant with shrub increases there between 2003 and 2010 (48). Moreover, ecosystem process models indicate increases in arctic tundra methane emissions matching sea-ice fluctuations and trend for the period from 1979 to 2006 (47). Projecting carbon dynamics in terrestrial systems with future sea-ice declines is, however, complicated by the unknown extent to which respiration may increase with warming (47). A recent link between sea-ice decline and the annual extent of tundra fires in Alaska (49) also suggests that ice loss may contribute to periodic massive pulses of terrestrial carbon release.

Future Challenges

Despite numerous examples of effects of declining sea ice on dynamics, abundance, and interactions among species, foreseeing the consequences of continued sea-ice loss remains difficult. A considerable challenge is to assign attribution, with greater certainty, to sea ice as a driver of ecological dynamics. The associations that we have drawn are weakened by reliance on patterns of covariation between sea-ice dynamics and ecological dynamics. Increasing emphasis on sea-ice decline as a contributing factor to regional warming (17) will improve the potential for increased recognition of sea-ice decline as a driver of ecological dynamics (4, 45). The field of joint attribution (50) in studies of ecological response to climate change can be informative here. Joint attribution is a statistical approach for assigning causation by anthropogenic forcing in recent warming and causation by warming in observed ecological dynamics (50). Further development and application of this approach will improve our ability to detect ecological responses to sea-ice decline.

A second challenge is to foresee and anticipate the human dimension as sea-ice decline increasingly facilitates access to coastal and near-shore areas for increased industrial development and extended-season shipping. In the Arctic, loss and thinning of sea ice is anticipated to increase accessibility of near-coastal and remote marine zones of all eight arctic nations by up to 28% by the middle of this century (51). Increased human access to formerly remote areas of the Arctic could have negative consequences for many species and their habitats, including those exploited by humans. Increased marine access will also likely accelerate the pace of arctic mineral and petroleum exploration in both terrestrial and marine systems (52), with increased threats to marine species such as bowhead whales (53) and Pacific walrus (51). Viewing sea ice as an important indicator of climatic warming and as an integrator and driver of ecological dynamics in the Arctic will improve our understanding of the systems-level functioning of this region and our basis for anticipating and responding to further change.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/341/6145/519/DC1
Fig. S1

10.1126/science.1235525

A Role for SIRT2-Dependent Histone H3K18 Deacetylation in Bacterial Infection

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Introduction: Posttranslational modification of histones is a well-documented mechanism by which the chromatin structure is modulated to regulate gene expression. Increasing evidence is revealing the strong impact of bacterial pathogens on host chromatin. However, our knowledge of the mechanisms underlying pathogen-induced chromatin changes and the impact of histone modifications and chromatin modifiers on infection is still in its infancy.

Methods: We used the model bacterium *Listeria monocytogenes* and analyzed the mechanisms underlying a specific histone modification, deacetylation of histone H3 on lysine 18 (H3K18). Through immunoblotting, mass spectrometry, and chromatin immunoprecipitation, we studied how infection affected this modification, both in vitro and in vivo. We used a combination of chemical inhibitors, small interfering RNA (siRNA), and knockout mice to discover the key role of the host histone deacetylase sirtuin 2 (SIRT2) and determine its effect on infection. We performed microarray analysis to identify how infection and SIRT2 modulated host transcription.

Results: *L. monocytogenes* induces deacetylation of H3K18. This modification is mediated by the host deacetylase SIRT2. Upon infection, SIRT2 translocates from the cytosol to the chromatin of the host at the transcription start sites of a subset of genes that are repressed. We find that this process is dependent on activation, by the bacterial protein InlB, of the cell surface receptor Met and downstream phosphatidylinositol 3-kinase (PI3K)/AKT signaling. Finally, infecting cells in which SIRT2 activity was blocked (by pharmacological agents, treatment with siRNA, or the use of SIRT2^{-/-} mice) resulted in a significant impairment of bacterial infection, showing that activity of SIRT2 is necessary for infection, both in vitro and in vivo.

Discussion: Our study identifies a stimulus, infection by *L. monocytogenes*, that leads to nuclear localization of SIRT2, a deacetylase previously shown to be mainly cytoplasmic. In fact, only upon infection and SIRT2 translocation from the cytoplasm to the chromatin does this deacetylase have a role in transcriptional repression. This mechanism of host subversion could be common to other invasive pathogens that induce deacetylation of histones, and it defines a target for potential therapeutic treatment.

FIGURES IN THE FULL ARTICLE

Fig. 1. Infection induces deacetylation of H3K18.

Fig. 2. Infection induces H3K18 deacetylation in vivo.

Fig. 3. The catalytic activity of SIRT2 is necessary for H3K18 deacetylation.

Fig. 4. SIRT2 translocates to the nuclear and chromatin fractions upon infection.

Fig. 5. H3K18 deacetylation and SIRT2 translocation is mediated by the cellular receptor Met.

Fig. 6. SIRT2 regulates gene transcription during infection.

Fig. 7. SIRT2 is essential for infection by *L. monocytogenes*.

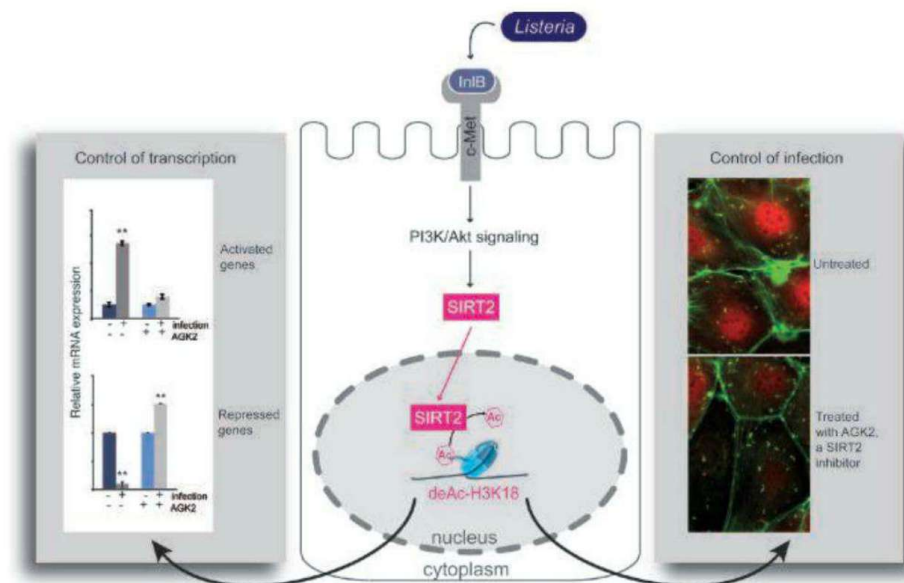
Fig. 8. The activity of SIRT2 is important for in vivo infection by *L. monocytogenes*.

SUPPLEMENTARY MATERIALS

Figs. S1 to S10
Tables S1 to S5

MULTIMEDIA RESOURCES

The transcriptome analysis data for this publication can be found in ArrayExpress with the accession number E-MEXP-3912.



Mechanism and consequence of SIRT2 activation by *L. monocytogenes*. *Listeria* induces SIRT2 relocalization from cytoplasm to chromatin, where SIRT2 deacetylates H3K18. The consequences of this cascade are control of host transcription, as illustrated by representative genes regulated by SIRT2, and control of infection, as assessed by staining cells for the secreted bacterial factor InlC (red), which is overexpressed in the cytosol, and host actin, which is polymerized into comet tails by bacteria (green). Error bars indicate SEM; ***P* < 0.001. Ac, acetyl; deAc, deacetylase.

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A Role for SIRT2-Dependent Histone H3K18 Deacetylation in Bacterial Infection

Haig A. Eskandarian,^{1,2,3} Francis Impens,^{1,2,3,4,5} Marie-Anne Nahori,^{1,2,3} Guillaume Soubigou,⁶ Jean-Yves Coppée,⁶ Pascale Cossart,^{1,2,3*} Mélanie A. Hamon^{1,2,3*}

Pathogens dramatically affect host cell transcription programs for their own profit during infection, but in most cases, the underlying mechanisms remain elusive. We found that during infection with the bacterium *Listeria monocytogenes*, the host deacetylase sirtuin 2 (SIRT2) translocates to the nucleus, in a manner dependent on the bacterial factor InlB. SIRT2 associates with the transcription start site of a subset of genes repressed during infection and deacetylates histone H3 on lysine 18 (H3K18). Infecting cells in which SIRT2 activity was blocked or using SIRT2^{-/-} mice resulted in a significant impairment of bacterial infection. Thus, SIRT2-mediated H3K18 deacetylation plays a critical role during infection, which reveals an epigenetic mechanism imposed by a pathogenic bacterium to reprogram its host.

Chromatin is a dynamic and highly regulated structure crucial for compacting DNA into the nucleus without compromising vital functions such as gene expression, cell differentiation, or cell division. The basic unit of chromatin is the nucleosome, which is composed of 147 base pairs of DNA wrapped around an octamer of histone proteins H2A, H2B, H3, and H4 (1). One well-documented mechanism by which the chromatin structure is modulated is posttranslational modification of histones (2). Each histone can be modified on different residues by phosphorylation, acetylation, methylation, etc. The cellular outcome of such modifications depends on the residue targeted and its surrounding context. Increasing evidence shows the impact of histone modifications on host immunity and bacterial pathogenesis (3, 4).

Acetylation and deacetylation are important histone modifications that regulate many cellular processes, including nucleosome assembly, chromatin compaction, and gene expression. Acetylation is mediated by histone acetyltransferases (HATs), which use acetyl-coenzyme A as a cofactor to catalyze the transfer of an acetyl group to a lysine residue, thereby reducing its charge (5). This modification lowers the affinity between histones and DNA, generally allowing chromatin to adopt a more relaxed structure and the transcription machinery to be recruited. Deacetylation, which is mediated by histone de-

acetylases (HDACs), counteracts the effects of acetylation and, in most cases, is associated with transcriptional repression. Despite their names, both HATs and HDACs have also been shown to target nonhistone proteins (6).

In humans, 18 HDACs are grouped into four classes. The 10 members of classes I (HDAC1, -2, -3, and -8) and II (HDAC4, -5, -6, -7, -9, and -10) share considerable similarity to each other in their catalytic core that uses zinc as a cofactor, but differ in size and structural organization (7). Class I is homologous to the yeast deacetylase RPD3 and class II with yeast HDA1 (8). HDAC11 is sometimes categorized as a class I member based on homology to RPD3, but phylogenetic analysis indicates that this deacetylase belongs to a separate class, referred to as class IV (9). Class III HDACs have no sequence similarity to classes I and II, are homologous to the yeast transcriptional repressor Sir2, and use nicotinamide adenine dinucleotide as a cofactor (10, 11).

The seven class III members, also called sirtuins, have been found in a wide variety of subcellular locations and are involved in different cellular processes (12). Human sirtuin 1 (SIRT1) localizes to the nucleus and has been shown to be involved in transcriptional repression (13). In contrast, the activities of SIRT2 and SIRT3 have been mainly characterized in extranuclear compartments, the cytosol and the mitochondria, respectively (10, 14). The role of HDACs and, more specifically, sirtuins in bacterial infections has not been investigated to date.

Listeria monocytogenes is a foodborne pathogen, which mainly causes disease in immunocompromised patients and pregnant women. Major advances in the understanding of infection have come from the characterization of the mechanisms by which this facultative intracellular pathogen invades host cells, evades killing,

and exploits cellular functions through the activity of its numerous virulence factors (15). The two proteins InlA and InlB promote entry into nonphagocytic cells. Once internalized, *L. monocytogenes* escapes the host vacuole by secretion of the pore forming toxin listeriolysin O (LLO) and phospholipases PlcA and PlcB (16). In the cytosol, *L. monocytogenes* grows and divides by exploiting host nutrients, including glucose-6-phosphate that is uptaken by the Hpt permease (17). *Listeria* perpetuates its intracellular lifestyle by polymerizing host actin using ActA and spreading to neighboring cells (15). To escape recognition, InlC dampens the host immune pathway NF- κ B, and InlK and ActA counteract autophagy (18–20).

Increasing evidence is uncovering the strong influence of bacterial pathogens on host chromatin (21–23). However, our knowledge of the impact of histone modifications and chromatin modifiers on infection is in its infancy. In this study, we reveal a central role of SIRT2 and deacetylation of H3 in the transcriptional reprogramming imposed by the bacterial pathogen *L. monocytogenes*. We show that SIRT2 can be targeted to the chromatin of host cells, where it deacetylates histone H3 on lysine 18 (H3K18) at a subset of genes. SIRT2 activity is crucial during a listerial infection, defining H3K18 deacetylation as a key mechanism that allows host subversion.

Results

L. monocytogenes Induces Deacetylation of Lysine 18 at the Histone H3 N-Terminal Tail

Our previous study of histone modifications induced during *L. monocytogenes* infection of host epithelial cells showed a deacetylation on the N-terminal tail of histone H3 (3). To characterize this infection-induced histone modification, our first aim was the identification of the lysine residue(s) that were deacetylated. Antibodies raised against specific H3 acetyl-lysine residues were used to assess the level of acetylation at lysines 9, 14, and 18 in cells infected with *L. monocytogenes*. Deacetylation was specifically observed to occur on H3K18 by 3 hours of infection of HeLa cells and continued to decrease through 24 hours of infection (Fig. 1A). Within the first 5 hours of infection, no deacetylation was observed at lysine 9 (K9), K14 of histone H3, or K16 of histone H4, suggesting that under the conditions tested, only K18 is modified during infection (Fig. 1A).

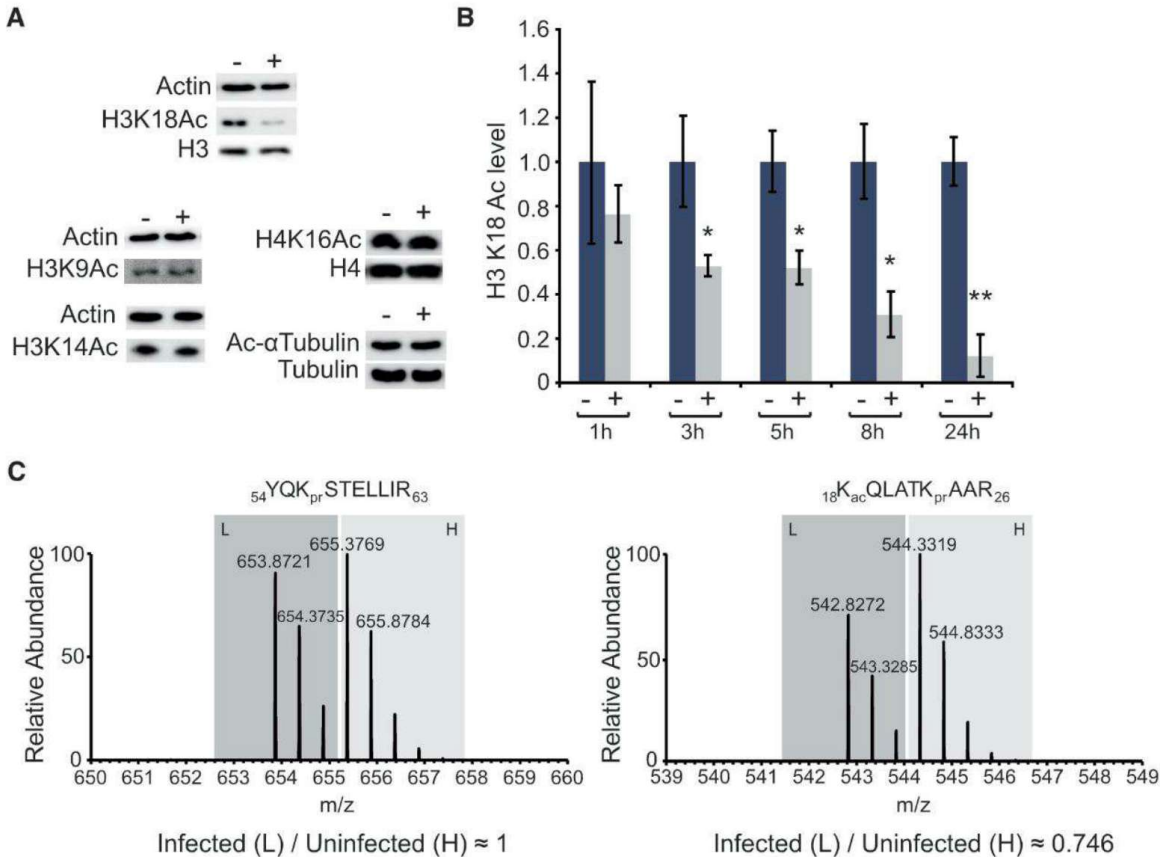
We had previously characterized two other histone modifications induced by *L. monocytogenes*—dephosphorylation of serine 10 on histone H3 and deacetylation of histone H4—showing that they were induced by one virulence factor, the toxin LLO (3). However, as shown in fig. S2, a mutant *Listeria* lacking the gene encoding LLO, Δ hly, induces the same deacetylation of H3K18 as a wild-type (WT) strain. Therefore, H3K18

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Fig. 1. Infection induces deacetylation of H3K18. (A) Acetylation levels in uninfected HeLa (-) and *L. monocytogenes*-infected cells (+), as detected by immunoblotting. (B) Quantification of acetylated H3K18 immunoblots in HeLa cells ($n \geq 3$ experiments). Error bars represent SEM. Statistical significance was calculated using a Student's *t* test. * $P < 0.05$; ** $P < 0.001$. (C) The peptides detected by MS are indicated above the graphs (62). YQK_{pr}STELLIR peptide, which is near the C terminus of histone H3 (pr, propionyl group), is used as control. The lysine residue at position 56 was exclusively identified in its light (L) ($m/z = 653.8721$, EGD) or heavy (H) ($m/z = 655.3769$, uninfected) propionylated form, indicating that this residue was not modified. The observed ion ratio is roughly 1/1, demonstrating equal mixing of both samples. The second peptide, K_{ac}QLATK_{pr}AAR (ac, acetyl group) carries acetylated K18 at its N terminus. Doublet peaks at m/z 542.8272 and 544.3319 correspond to forms of the peptide with light or heavy propionylated K23, derived from the infected and uninfected sample, respectively. The lower



presence of this peptide in the infected sample indicated deacetylation of K18 with 25.4%. The ratio of light peptides (corresponding to the infected sample) to the heavy peptides (the uninfected sample) is shown under the graph ($n = 2$).

deacetylation is independent of the histone modifications previously observed in *Listeria* and occurs through a different mechanism that we characterize below.

Deacetylation of H3K18 was then assessed in vivo. The spleens of Balb/c mice were collected after intravenous infection with *L. monocytogenes* and compared with those of uninfected mice. Similar to what is observed in in vitro infection, deacetylation of H3K18, but not H3K9 or H3K14, occurred in the spleens harvested after 72 and 96 hours of infection (Fig. 2, A and B).

SIRT2 Is Necessary for H3K18 Deacetylation During Infection

To identify the host factor involved in infection-induced H3K18 deacetylation, we blocked the activity of HDAC classes with specific chemical inhibitors and tested their effect on H3K18 acetyl levels in infected cells. The activity of HDAC classes I and II was blocked with trichostatin A (TSA), and that of class III, the sirtuins, was blocked with nicotinamide (NIC). TSA treatment did not inhibit infection-induced deacetylation, whereas NIC treatment completely blocked H3K18 deacetylation, suggesting a role for sirtuins in deacetylation (fig. S3A). Next, we used specific inhibitors of the sirtuin family. Whereas a

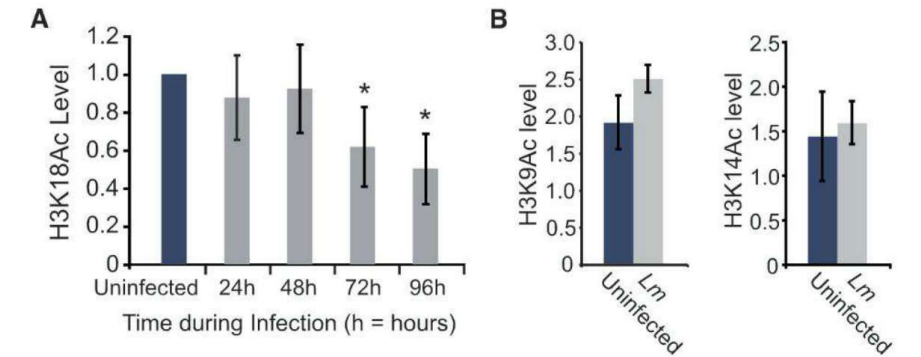


Fig. 2. Infection induces H3K18 deacetylation in vivo. (A) Balb/c mice were sacrificed at the indicated times after the start of infection. The spleens were collected, and the level of acetylated H3K18 was analyzed by immunoblot. (B) Quantification of H3K9 and H3K14 acetylation levels in spleens of mice uninfected or infected with *L. monocytogenes* (Lm) for 72 hours. $n \geq 4$ mice per time point. Error bars indicate SEM. Statistical significance was calculated using a Student's *t* test. * $P < 0.05$; ** $P < 0.001$.

SIRT1 inhibitor, 6-chloro-2,3,4,9-tetrahydro-1H-carbasole-1-carboxamide (CTCC), had no effect, a SIRT2 inhibitor, 2-cyano-3-[5-(2,5-dichlorophenyl)-2-furanyl]-N-5-quinolinyl-2-propenamide (AGK2), blocked infection-induced deacetylation of H3K18 (Fig. 3A). With the use of small interfering RNA (siRNA), we also knocked down SIRT1, SIRT2, SIRT6, or SIRT7, which have reported deacetylase

activity and are localized either in the cytoplasm or nucleus. In agreement with results obtained with chemical inhibitors, only the SIRT2 siRNA blocked H3K18 deacetylation upon infection, suggesting that SIRT2 is the HDAC responsible for this modification (Fig. 3B).

To definitively determine that the catalytic activity of SIRT2 was necessary for H3K18 de-

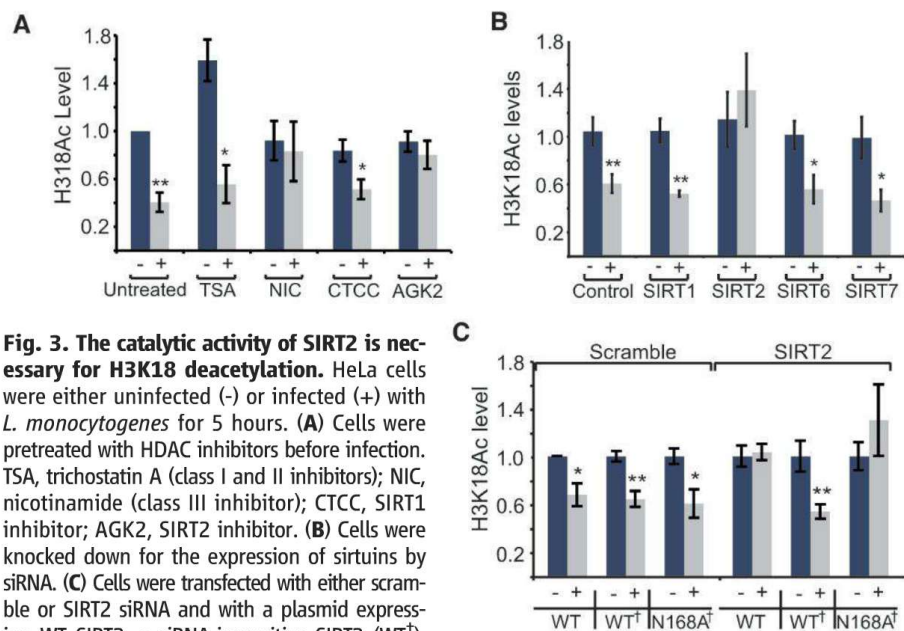


Fig. 3. The catalytic activity of SIRT2 is necessary for H3K18 deacetylation. HeLa cells were either uninfected (-) or infected (+) with *L. monocytogenes* for 5 hours. (A) Cells were pretreated with HDAC inhibitors before infection. TSA, trichostatin A (class I and II inhibitors); NIC, nicotinamide (class III inhibitor); CTCC, SIRT1 inhibitor; AGK2, SIRT2 inhibitor. (B) Cells were knocked down for the expression of sirtuins by siRNA. (C) Cells were transfected with either scramble or SIRT2 siRNA and with a plasmid expressing WT SIRT2, a siRNA-insensitive SIRT2 (WT[†]), or a catalytically inactive siRNA-insensitive SIRT2 (N168A[†]). H3K18 acetylation levels were quantified by immunoblot ($n \geq 3$). Error bars denote SEM. Statistical significance was calculated using a Student's *t* test. * $P < 0.05$; ** $P < 0.001$.

acetylation during infection, we complemented cells knocked down for SIRT2 by siRNA by using two plasmids expressing the wild type or a catalytically inactivated SIRT2 [Asn¹⁶⁸→Ala¹⁶⁸ (N168A)] (24) insensitive to siRNA. Transfecting SIRT2 knockdown cells with a siRNA-insensitive SIRT2 resulted in H3 deacetylation upon infection (Fig. 3C). This effect was observed only when transfecting with the siRNA-insensitive WT SIRT2. Deacetylation did not occur when complementing cells with the siRNA-insensitive N168A SIRT2. Furthermore, deacetylation occurred normally upon infection in control experiments using scramble siRNA, showing that N168A SIRT2 is not dominant negative (Fig. 3C). Thus, the deacetylase activity of SIRT2 is important for H3K18 deacetylation during infection.

SIRT2 Is Targeted to the Nucleus and Is Associated to Chromatin upon Infection

To determine the site of action of SIRT2, we examined its localization during infection. The activity of SIRT2 has mainly been characterized in the cytoplasm of resting cells (25). We first assessed the subcellular localization of endogenous SIRT2 by immunofluorescence. Although SIRT2 in uninfected cells was detected in both the cytosol and the nucleus, cells infected with *L. monocytogenes* showed a clear nuclear labeling, suggesting that infection induces relocalization of this protein (Fig. 4A).

The subcellular localization of SIRT2 was further characterized by cell fractionation. Cells were fractionated into cytosolic, nuclear soluble, and chromatin fractions. Control proteins known to localize to these cellular compartments were used to confirm the purity of each cell fraction.

Detection of SIRT2 in these cellular fractions showed that SIRT2 (the two major splice variants) was present in the cytosol and in the soluble nuclear fraction in both uninfected and infected cells. Notably, in infected cells, SIRT2 was mostly detected in the chromatin fraction, where H3K18 deacetylation was observed (Fig. 4B). It is important to note that the activity of SIRT2 appears to be restricted to the chromatin fraction, as the cytoplasmic target tubulin was not deacetylated (Fig. 1A).

In parallel to immunoblotting cell fractions for SIRT2, we immunoprecipitated SIRT2 from each of the cytosolic, nuclear soluble, and chromatin fractions and performed mass spectrometry (MS) on the recovered material. The results confirmed that there was 10- to 20-fold more SIRT2 in the chromatin and nuclei of infected cells compared with that of uninfected cells (Fig. 4C).

We further assessed whether retaining SIRT2 in the nucleus with leptomycin B (without infection) was sufficient to deacetylate H3K18. Leptomycin B caused SIRT2 accumulation in the nucleus but did not lead to H3K18 deacetylation (fig. S3, A and B). In addition, a catalytically inactive SIRT2, which relocalized to the nucleus upon infection, did not induce H3K18 deacetylation (Fig. 3C and fig. S3C). Thus, infection induces activation and targeting of SIRT2 to the chromatin fraction where deacetylation of H3K18 occurs.

H3K18 Deacetylation and SIRT2 Nuclear Targeting Are Triggered by the *Listeria* Virulence Factor, InlB

To identify the bacterial factor(s) necessary for inducing H3K18 deacetylation, we screened

L. monocytogenes mutants defective for infection. One mutant, Δ *InlB*, which is defective for invasion of HeLa cells, did not exhibit H3K18 deacetylation, suggesting that either the InlB protein itself or the entry of bacteria into cells is important for inducing H3K18 deacetylation (Fig. 5A). InlB is a well-expressed surface protein of *Listeria* that, upon interaction with the cell-surface receptor c-Met, mediates entry of bacteria or beads into nonphagocytic cells. We tested whether a noninvasive species, *L. innocua* (which, when engineered to express InlB, is able to enter into HeLa cells), can induce H3K18 deacetylation. Although *L. innocua* had no effect, *L. innocua* expressing InlB led to H3K18 deacetylation levels similar to those induced by *L. monocytogenes* (Fig. 5A), suggesting that no other virulence factor besides InlB is required for H3K18 deacetylation. Similar results were obtained with polystyrene beads coated with InlB, which induced H3 deacetylation, whereas uncoupled beads had no effect (Fig. 5A). Furthermore, purified InlB was sufficient to induce H3K18 deacetylation (Fig. 5A). When treating cells with the natural c-Met ligand hepatocyte growth factor (HGF), deacetylation was also observed to similar levels and with the same kinetics as with purified InlB (Fig. 5A and fig. S4A). In contrast, cells treated with epidermal growth factor (EGF), which binds the EGF receptor, had no effect (Fig. 5A and fig. S4A). The correlation between deacetylation of H3K18 and SIRT2 relocalization was also assessed with purified InlB, HGF, and EGF. Nuclear accumulation of SIRT2 was observed in all conditions where deacetylation occurred; accumulation did not occur upon EGF treatment (Fig. 5B). Thus, through the c-Met receptor, InlB or HGF are sufficient to induce SIRT2 nuclear translocation and H3K18 deacetylation.

Met-Induced PI3K/Akt Signaling Is Necessary for H3K18 Deacetylation and SIRT2 Nuclear Recruitment

We next addressed the effect of the signaling cascade downstream of c-Met on H3K18 deacetylation. We treated cells with inhibitors of tyrosine phosphorylation, phosphatidylinositol 3-kinase (PI3K), and Akt, all known to be activated upon binding of InlB to c-Met, and assessed acetyl H3K18 levels and SIRT2 relocalization. When cells were treated with genistein to block tyrosine phosphorylation, neither InlB nor HGF induced H3K18 deacetylation or SIRT2 nuclear targeting within the time frame assayed (Fig. 5B and fig. S4A). To control for the activity of genistein, we monitored levels of P-Akt, a downstream target of the c-Met-dependent tyrosine phosphorylation. Although InlB and HGF induced an increase in the levels of phospho-Akt, this decrease was not observed in samples treated with genistein (fig. S4D).

Next, we used either chemical inhibition of PI3K, with wortmannin or LY294002, or expression of a dominant-negative p85 regulatory subunit to show that PI3K activity was necessary for

SIRT2 nuclear accumulation and H3K18 deacetylation (Fig. 5B and fig. S4, A and B). We further assessed the role of Akt by using the chemical inhibitor 1-L-6-hydroxymethyl-chiro-inositol 2-(R)-2-methyl-3-O-octadecylcarbonate (HIMO). This compound blocked InlB- and HGF-dependent H3K18 deacetylation and nuclear relocalization of SIRT2 (Fig. 5B and fig. S4C). Together, these results establish that the signaling cascade mediated by the cell receptor c-Met and the downstream signaling factors PI3K and Akt is one essential pathway linking *L. monocytogenes* to SIRT2 and H3K18 deacetylation.

L. monocytogenes Infection Induces SIRT2-Dependent Modulation of Host Gene Expression

To further characterize the role of SIRT2 during infection, we searched for genes modulated during infection in a SIRT2-dependent manner. Transcriptome analyses were carried out comparing four different conditions: uninfected HeLa cells, cells infected for 5 hours with *L. monocytogenes*, and AGK2 pretreated cells with or without infection. When comparing uninfected cells, treated or not with AGK2, we did not identify any genes that were differentially regulated in a significant manner, suggesting that SIRT2 has no effect on resting cells (Fig. 6A). This observation is consistent with our cell fractionation data, which show that in resting cells only a small fraction of SIRT2 is bound to chromatin. In contrast, AGK2 had a significant effect on gene transcription induced by infection. In the absence of AGK2, infection with *L. monocytogenes* led to activation of 158 genes and repression of 272 genes (table S5). Notably, pretreatment with AGK2 significantly decreased the number of infection-induced activated and repressed genes to 30 and 1, respectively (Fig. 6A). Using these data, we categorized genes as SIRT2-independent if AGK2 pretreatment had no effect on their expression and SIRT2-dependent if AGK2 affected their expression, and we validated these results by quantitative polymerase chain reaction (PCR) on a subset of genes (fig. S5). Because AGK2 displayed the most significant effect on repressed genes and because most genes repressed during infection are dependent on SIRT2 for their expression pattern, we conclude that SIRT2 has a key role in gene repression during infection.

We further tested whether the genes identified by transcriptome analysis were similarly regulated by HGF and the bacterial factor InlB, as with infection. Both treatments with InlB and HGF resulted in the same down-regulation of genes identified in the SIRT2-dependent signature (fig. S6). These results suggest that *L. monocytogenes* is hijacking SIRT2 to impose a transcriptional control on the host, and this response is mediated by the Met receptor.

H3K18 Deacetylation and SIRT2 Recruitment Occur at the TSS of SIRT2-Regulated Genes

Previous studies in T cells have shown that H3K18 acetylation levels are enriched at transcriptional

start sites (TSSs) and enhancers of active mammalian genes (26, 27). We thus probed the TSSs of SIRT2-regulated genes for SIRT2 recruitment and H3K18 deacetylation by chromatin immunoprecipitation (ChIP). Whereas SIRT2-independent and -dependent activated genes exhibited an increase in H3K18 acetylation levels and a loss of SIRT2 at transcription start sites, SIRT2-dependent repressed genes showed an opposite phenotype (Fig. 6, B and C). All tested SIRT2-dependent repressed genes (*MYLIP*, *EHHADH*, *SYDE2*, *ERCC5*, and *LEF1*) exhibited a more than 10-fold increase in recruitment of SIRT2 and a significant decrease in the level of acetylated H3K18 upon infection (Fig. 6C). SIRT2 recruitment and H3K18 deacetylation were observed only at the TSSs and not at exon 2, strongly supporting a role for SIRT2 in transcriptional regulation (fig. S7). We further verified that other histone residues were not modified upon SIRT2 recruitment. ChIP experiments with antibodies against AcH3K9, AcH3K14, and AcH4K16 (anti-AcH3K9, anti-AcH3K14, and anti-AcH4K16,

respectively) showed that none of the corresponding residues were deacetylated at the genes where SIRT2 was recruited (fig. S8). Because the histone acetyl transferase CBP is displaced by infection with adenovirus, which culminates in a threefold reduction in cellular H3K18 acetylation, we asked whether the same mechanism was acting during a *Listeria* infection. Therefore, we verified whether CBP was being relocalized by infection with *L. monocytogenes* as with adenovirus (28). There was no change in the level of CBP binding at SIRT2-repressed genes, where H3K18 deacetylation occurs (fig. S8). Therefore, our data support a model in which infection targets SIRT2 to a subset of genes, where it specifically imposes H3K18 deacetylation and gene repression.

SIRT2 Is Critical for Infection with L. monocytogenes

We next assessed the impact of SIRT2 on infection. We first used either a siRNA approach or a pharmacological approach to perform experi-

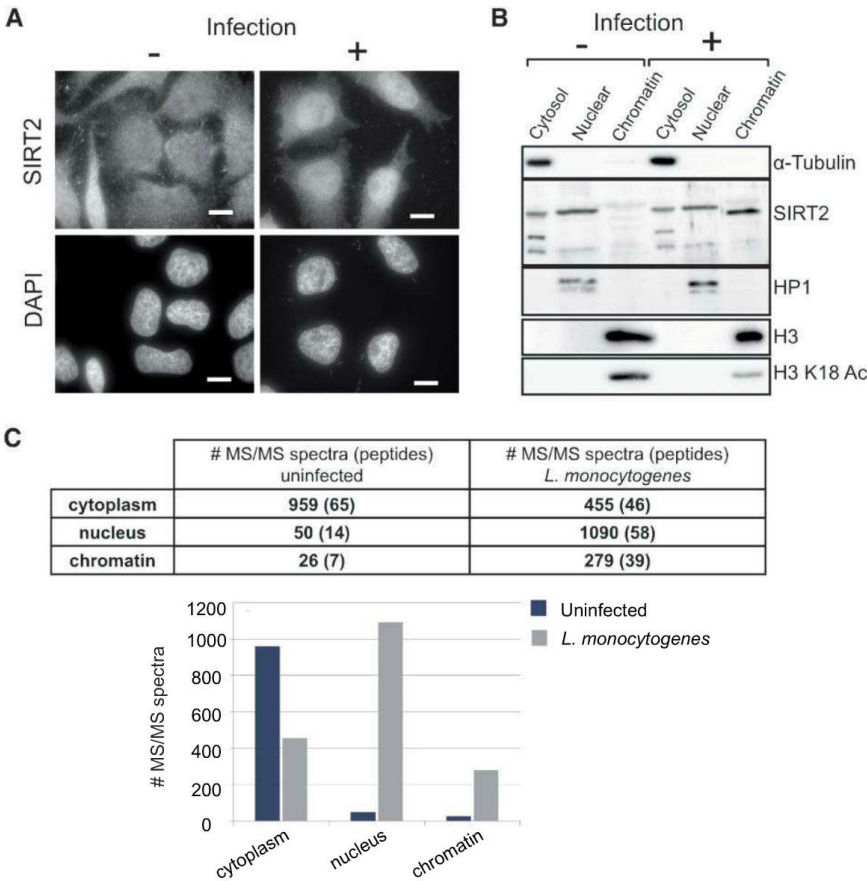


Fig. 4. SIRT2 translocates to the nuclear and chromatin fractions upon infection. (A) Endogenous SIRT2 was detected by immunofluorescence in HeLa cells uninfected (-) or infected (+) for 5 hours. Scale bars, 10 μ m. DAPI, 4',6-diamidino-2-phenylindole. (B) Uninfected cells (-) or 5-hour infected cells (+) were fractionated and immunoblotted for the indicated proteins. Experiments represent $n \geq 3$. (C) Immunoprecipitation of SIRT2-FLAG from cytosolic, nuclear, and chromatin fractions was analyzed by MS. The numbers of SIRT2 fragments detected by MS/MS are indicated in the table; numbers of the corresponding SIRT2 peptides are in parentheses. A graphical representation of the MS/MS spectra is also shown.

ments in tissue culture cells. Infection was quantified by either immunoblot—measuring the levels of a secreted bacterial factor, InlC, which accumulates during infection (18)—or fluorescence-activated cell sorting (FACS) analysis of host cells infected with green fluorescent protein (GFP)-expressing *L. monocytogenes*. Treating cells with siRNA against SIRT1, -6, or -7 or a SIRT1 chemical inhibitor, CTCC, had no effect on infection (Fig. 7, A and B). In contrast, cells treated with a SIRT2 siRNA or an inhibitor, AGK2, were significantly less infected than untreated cells (Fig. 7, A and B). AGK2 is not toxic to bacteria, nor does it affect the host's cell cycle (fig. S9). Although the initial stages of infection progressed similarly in the presence or absence of AGK2, at later times of infection, the levels of InlC or the GFP fluorescence were significantly greater in untreated cells than in cells treated with AGK2 (Fig. 7A). Furthermore, *Listeria* comet tails were not affected by AGK2 treatment, suggesting that loss of SIRT2 activity has no effect on motility (Fig. 7C and fig. S10). In addition, SIRT2 activity was important for infection of the listerial mutant Δ actA, which is defective in cell-to-cell spreading (Fig. 7A). Thus, SIRT2 is required for the late stages of a listerial infection, probably for bacterial replication, in cultured cells.

The impact of SIRT2 on infection was also determined in vivo in *Sirt2*^{tm1a(EUCOMM)Wtsi} mice (*Sirt2*^{-/-}) generated at the Sanger Institute. WT or *Sirt2*^{-/-} mice were infected intravenously with *L. monocytogenes* and the spleens and livers were collected for bacterial enumeration. In agreement with our in vitro data, the spleens of *Sirt2*^{-/-} mice were significantly less infected than those of WT mice, confirming the crucial role of SIRT2 on infection in vivo (Fig. 8A). We further tested the role of InlB by comparing infection of a Δ inlB mutant in both WT and *Sirt2*^{-/-} mice. In contrast to WT *L. monocytogenes*, a Δ inlB mutant infected both WT and *Sirt2*^{-/-} mice strains similarly (Fig. 8A), confirming the importance of InlB in hijacking SIRT2 in vivo. We further assessed the levels of H3K18 acetylation in the spleens of infected mice. Whereas deacetylation took place in WT mice, it did not occur in *Sirt2*^{-/-} mice or in WT mice infected with a Δ inlB mutant (Fig. 8B). Thus, activity of SIRT2 on H3K18 is important for infection in vivo, and InlB is the bacterial factor triggering this activity.

Discussion

Here, we have shown that *L. monocytogenes* hijacks the host HDAC, SIRT2, to impose a transcriptional program on the host. In addition, we have uncovered a nuclear function for SIRT2

in deacetylating H3 specifically on lysine 18 in response to infection and to activation of the PI3K/AKT signaling cascade.

Histone Deacetylation upon Infection

The study of pathogen-induced histone modifications is a recent field of research. Three bacteria were reported to modulate the levels of acetylated histones during infection, but the underlying mechanisms have remained unknown. During a *Mycobacterium tuberculosis* infection, histone deacetylation occurs at the promoters of specific genes—*HLA-DR α* , *HLA-DR β* , and *CIITA*—correlating with transcriptional repression of these genes (29, 30). Infection of gastric epithelial cells by *Helicobacter pylori* was shown to induce deacetylation of H3K23 but had no effect on H3K9, H3K14, H3K18, H3K23, H3K9K14, or H4K8 (31). Finally, *Anaplasma phagocytophilum* was reported to activate the expression of genes encoding HDAC1 and -2, correlating with transcriptional repression of key immunity genes and a decrease in a general H3 acetylation levels at the promoter of these same genes (32).

Through binding and sequestering the HATs CBP and p300 to a specific subset of host genes, adenovirus infection causes a threefold reduction in total cellular histone H3K18 acetylation (28). The resulting effect was to stimulate cell cycling

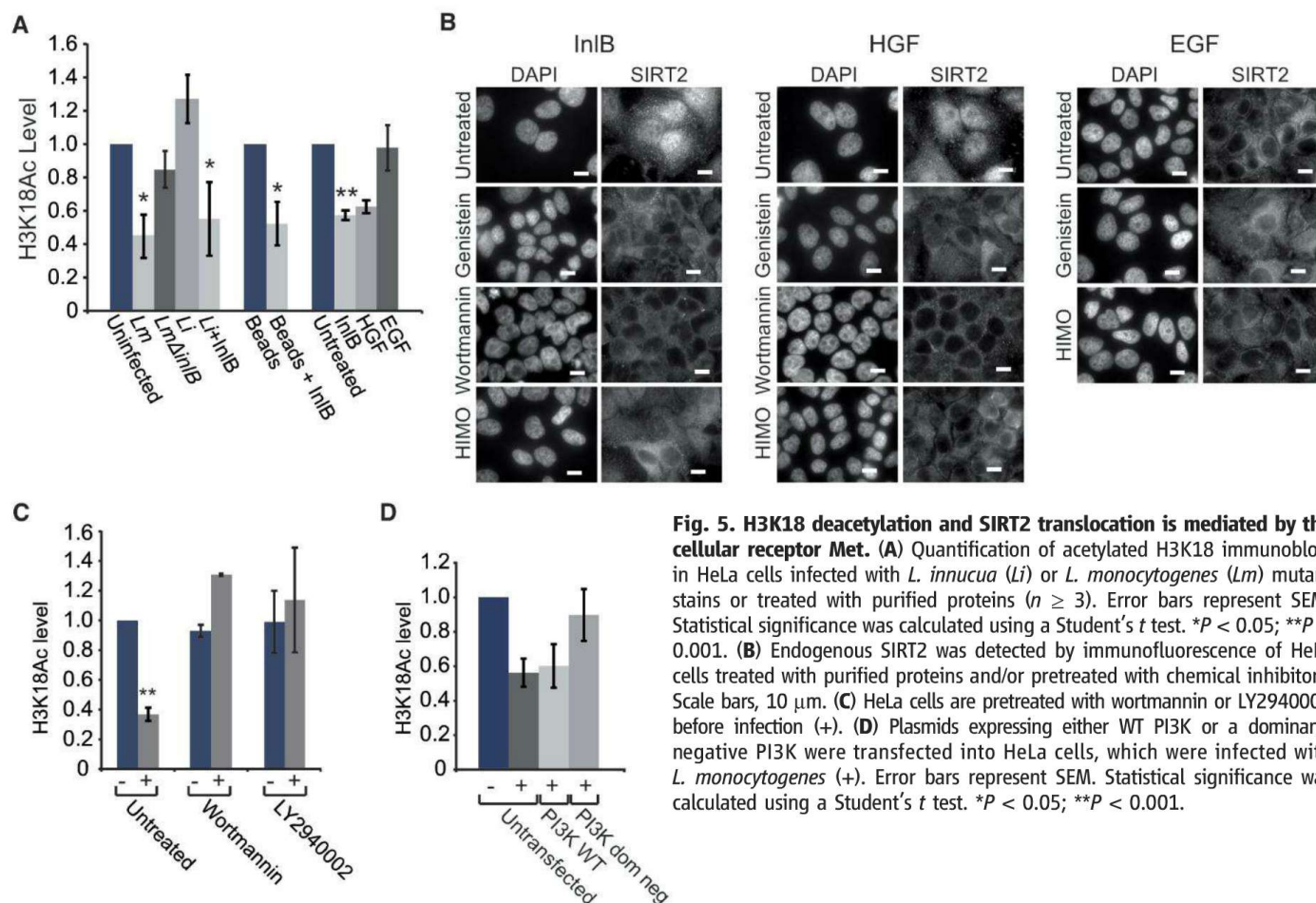


Fig. 5. H3K18 deacetylation and SIRT2 translocation is mediated by the cellular receptor Met. (A) Quantification of acetylated H3K18 immunoblots in HeLa cells infected with *L. innocua* (Li) or *L. monocytogenes* (Lm) mutant stains or treated with purified proteins ($n \geq 3$). Error bars represent SEM. Statistical significance was calculated using a Student's *t* test. * $P < 0.05$; ** $P < 0.001$. (B) Endogenous SIRT2 was detected by immunofluorescence of HeLa cells treated with purified proteins and/or pretreated with chemical inhibitors. Scale bars, 10 μ m. (C) HeLa cells are pretreated with wortmannin or LY2940002 before infection (+). (D) Plasmids expressing either WT PI3K or a dominant-negative PI3K were transfected into HeLa cells, which were infected with *L. monocytogenes* (+). Error bars represent SEM. Statistical significance was calculated using a Student's *t* test. * $P < 0.05$; ** $P < 0.001$.

and inhibit antiviral responses and cellular differentiation (33). Although the mechanism of H3K18 deacetylation appears different for adenovirus and *Listeria*, the cellular outcome could be the same. Thus, together with our results, these studies point to histone deacetylation as a common strategy used by pathogens during infection.

An Additional Nuclear Function for SIRT2

The role of SIRT2 has mainly been characterized in the cytoplasm, where it regulates microtubule dynamics through deacetylation of α -tubulin (34) and NF- κ B gene expression through deacetylation of p65 (35) and also controls adipocyte differentiation (36) and autophagy (37) through deacetylation of FOXO1. However, recent studies have identified nuclear SIRT2 targets such as p53 and p300 (34).

SIRT2-dependent deacetylation of H3K56 was shown to occur after DNA-damage-induced hyperacetylation (38). Additionally, in vitro studies have demonstrated that SIRT1, -2, and -3 can deacetylate H3K18 and H4K16 (39). The apparent contradiction of a protein being cytoplasmically located yet exhibiting specificity for a histone residue was resolved when SIRT2 localization was monitored during cell cycle progression. SIRT2 localizes to the cytoplasm throughout the cell cycle, with the exception of prophase at the beginning of M phase, where it translocates to the nucleus to cause deacetylation of H4K16 (40). However, in interphase cells, only one report describes an equilibrium between nuclear import and export of SIRT2, suggesting a constant shuttling of this protein (24). Our study identifies a

stimulus leading to nuclear localization of SIRT2 in interphase cells. Notably, our transcriptome analysis demonstrates that SIRT2 does not regulate transcription in resting cells and plays a role only during infection. Indeed, only in infected cells, in which SIRT2 is recruited to the host chromatin, does SIRT2 have a role in transcriptional repression.

The mechanism of SIRT2 localization to the nucleus remains unknown. We noticed a shift in the migrating band size of SIRT2 upon infection-induced localization to the chromatin (Fig. 4B), suggesting a posttranslational modification of SIRT2. Phosphorylation of SIRT2 has been reported: SIRT2 is phosphorylated at serine 368 by the CDK1 kinase and dephosphorylated by the CDC14A and B phosphatases (41). A smaller

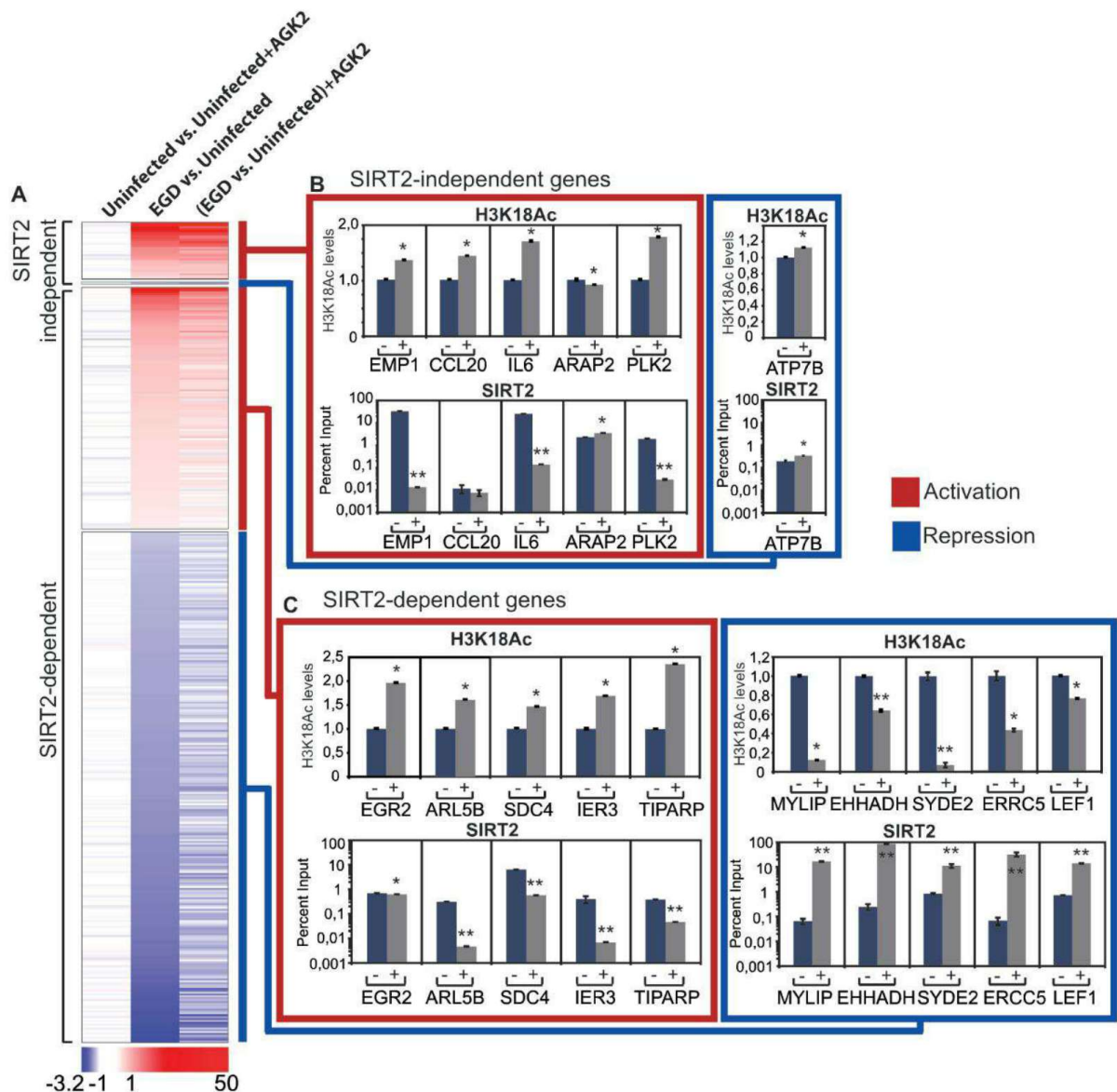


Fig. 6. SIRT2 regulates gene transcription during infection. (A) Heatmap representation of the mean fold change in gene expression, as determined by transcriptome analysis of Caco2 cells infected for 5 hours ($n \geq 2$). Red represents gene activation; blue indicates gene repression. (B and C) ChIP using

antibodies targeting SIRT2, H3K18Ac, and H3 was quantified by qPCR ($n \geq 3$). H3K18Ac qPCRs are normalized to H3 qPCRs, and SIRT2 qPCR results are represented as percent of the input. Error bars denote SEM. Statistical significance was calculated using a Student's t test. * $P < 0.05$; ** $P < 0.001$.

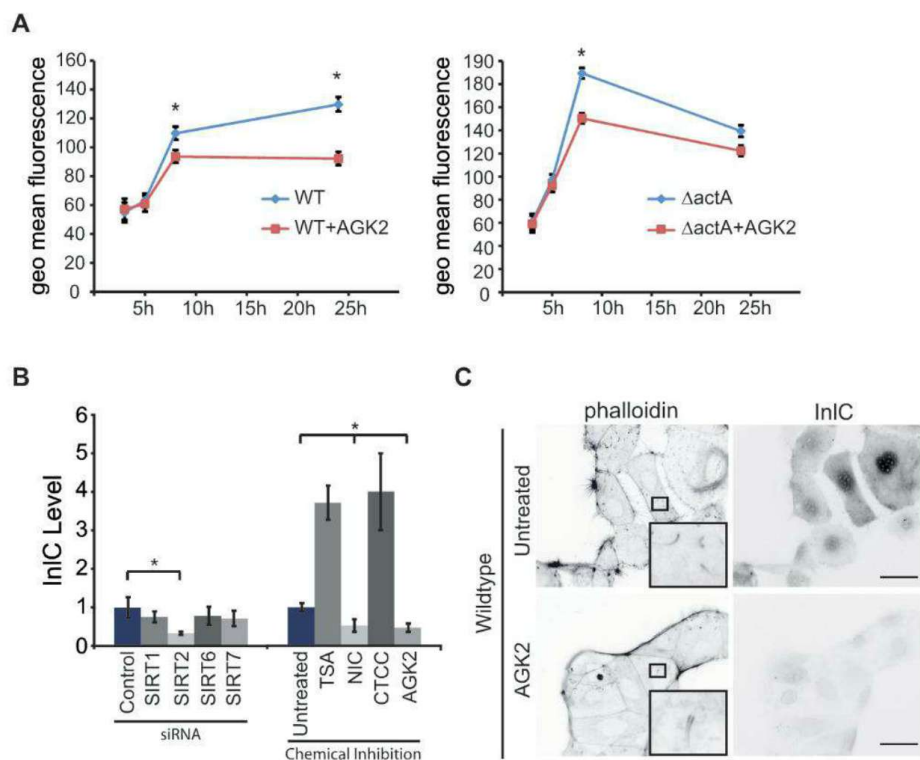


Fig. 7. SIRT2 is essential for infection by *L. monocytogenes*. (A) The level of *L. monocytogenes* infection is measured by FACS analysis of Caco2 cells pretreated (or not) with AGK2, where the geometric mean (y axis) represents the number of intracellular bacteria (10,000 cells measured; $n \geq 3$) at 3, 5, 8, and 24 hours after the start of infection. Error bars denote SEM. * $P < 0.05$, as measured with a Student's t test. (B) Quantification of immunoblots detecting the bacterial protein InIC in 5-hour-infected HeLa cells knocked down for SIRT1, 2, 6, and 7 by siRNA or in cells treated with chemical inhibitors to deacetylases. Error bars indicate SEM. Statistical significance was calculated using a Student's t test. * $P < 0.05$; ** $P < 0.001$. (C) Caco2 cells were treated (or not) with AGK2 and infected with WT *L. monocytogenes* for 5 hours. *Listeria* comet tails are visualized by fluorescence imaging of phalloidin and InIC by immunofluorescence (see text). Images are shown as negatives for better visualization. Scale bars, 50 μ m. Large inserted boxes show 5 $\times$ digital magnifications of the smaller boxes.

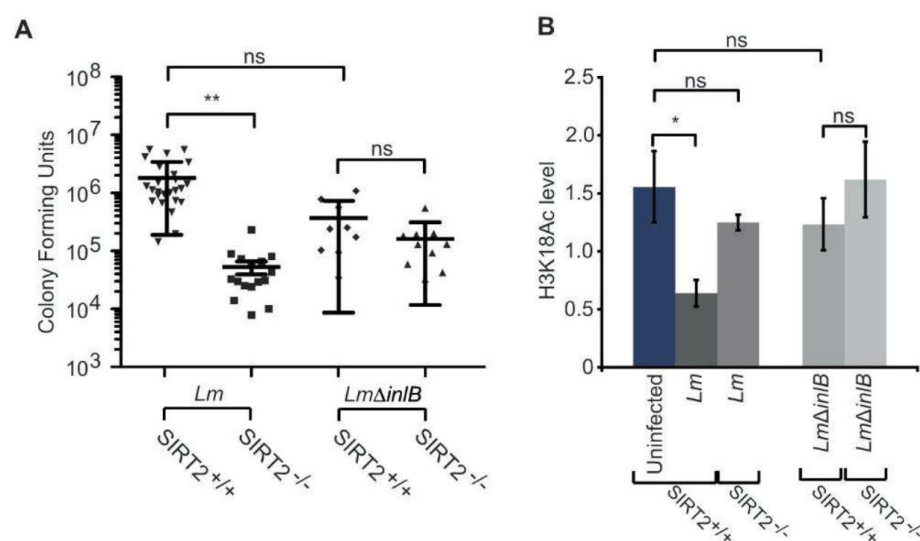


Fig. 8. The activity of SIRT2 is important for in vivo infection by *L. monocytogenes*. (A) Colony forming units in spleens of SIRT2^{+/+} or SIRT2^{-/-} mice infected for 72 hours with *L. monocytogenes* (Lm) or $\Delta inlB$. Each symbol represents one mouse. (B) Immunoblot analysis of H3K18 acetylation levels in mice spleens. Error bars indicate SEM. Statistical significance was calculated using a Student's t test. * $P < 0.05$; ** $P < 0.001$; ns, not significant.

SIRT2 isoform is phosphorylated by CDK2 and -5, at the serine residue corresponding to the Ser³⁶⁸ residue of the full-length protein described above (42). Although these phosphorylation events have been shown to affect cell cycle progression and enzymatic activity, no difference in SIRT2 subcellular localization has been reported. Our results show that infection with *Listeria* provides a practical stimulus that we can use to study the posttranslational modification of SIRT2 and its role in SIRT2 translocation.

PI3K/AKT Signaling and SIRT2

We have shown that the cellular receptor c-Met is important for inducing SIRT2 translocation to the nucleus. We further identified PI3K and AKT as downstream signaling intermediates to H3K18 deacetylation. To date, only one study has correlated AKT signaling with histone modifications (43). In this study, they demonstrate that activation of PI3K/AKT signaling can be a trigger for H3K27 trimethylation at many downstream target genes. The genes marked with H3K27 are different from those identified here, suggesting that the listerial response is different, even though it is mediated by PI3K/AKT. The difference in the signaling response terminating in H3K18 deacetylation is additionally illustrated by our observation that EGF, which also activates PI3K and AKT, does not induce H3 deacetylation or SIRT2 nuclear localization. Furthermore, both AKT signaling and H3K18 deacetylation are correlated with cancer development. The PI3K signaling cascade is one of the most commonly activated signaling pathways in human cancer, and H3K18 is correlated with aggressive cancer phenotypes and poor patient prognosis (44). However, although both PI3K activation and H3K18 deacetylation occur during infection with *L. monocytogenes*, oncogenic transformation has not been reported after listeriosis. SIRT2 has been described as having tumor-suppressing activities (45, 46). Together, additional factors that remain to be identified are important for providing specificity to the response induced by a *L. monocytogenes* infection.

Gene Regulation by SIRT2

Although many histone deacetylases have a role in transcriptional regulation, no such role has been directly attributed to SIRT2, as it has not previously been shown to target histones in vivo. Here, we demonstrate a role for SIRT2 in transcriptional regulation, because every down-regulated gene (except one) identified during infection is dependent on SIRT2 for repression. However, because H3 deacetylation is observed globally by immunoblot, we predict that it must also occur in other regions besides the TSSs of specific genes, perhaps in intergenic regions as reported for adenovirus infections (47).

A gene ontology analysis of the 271 repressed genes (listed in table S5) revealed that a significant number of them are DNA binding proteins (51 genes) and/or are implicated in transcriptional regulation (55 genes). A few prominent examples

are SMAD1 and FOXM1, transcription factors that participate in a wide range of critical cellular processes, including proliferation, differentiation, and apoptosis; IRF2, a transcription factor important in regulating the interferon response in immune response to infection; SMARCA2, which is a member of the SWI/SNF family of proteins that alter the chromatin structure in an adenosine triphosphate-dependent manner; and SAP130, which is part of the Sin3A repressor complex important in transcriptional repression. Thus, transcriptional remodeling by SIRT2 could be greater than what we report here, because a second wave of transcriptional modulation could follow the SIRT2-dependent deregulation of genes involved in transcriptional regulation. A significant number of SIRT2 down-regulated genes are involved in immune response regulation. For instance, many genes (such as *RASGRP1*, *MAPK14*, *PIK3R3*, *PTPBG*, *SOS1*, *VAV3*, *ABL1*, *CAMK26*, *MAP2K6*, and *LEF1*) are regulators of B and T cell receptor signaling. The chemokine Cxcl12, which is strongly chemotactic for lymphocytes, and the interferon transcription factor IRF2 are also down-regulated. From these data, we suggest that *L. monocytogenes* is hijacking SIRT2 to impose a transcriptional control of the host, thereby manipulating many essential cellular functions to promote a listerial infection.

Ingenuity analysis of our Affymetrix (Santa Clara, California) arrays showed that SIRT2 regulates a cluster of 36 genes that increase cell survival. The intracellular lifestyle of *L. monocytogenes* is consistent with an active mechanism to promote survival of the host cell. *Shigella flexneri* and *H. pylori* have been shown to dampen rapid turnover of epithelial cells to prolong colonization within the intestinal epithelial cells (48, 49). Our results suggest that this might also be the case for *Listeria*.

Role of SIRT2 During Infection

We have shown the importance of SIRT2 in infection, both in tissue culture cells and in vivo. Furthermore, we have demonstrated that SIRT2 plays an essential role in deacetylating H3K18. Its effect on H3K18 during infection appears to be specific, as deacetylation of other known targets such as tubulin and H4K16 does not occur (Fig. 1A). We thus suggest that the main role of SIRT2 in infection is to deacetylate H3K18; however, we cannot exclude that SIRT2 is targeting other proteins during infection, in addition to H3K18. Investigations are in progress to address this issue.

H3K18 was recently found to be deacetylated by SIRT7 in cancer cells (50), suggesting that different deacetylases act on this residue under different conditions.

In summary, our study provides evidence that *L. monocytogenes* hijacks the host HDAC, SIRT2, to impose a transcriptional program on the host through activation of the PI3K/AKT signaling cascade. We have, therefore, uncovered a nuclear function for SIRT2 in deacetylating H3

specifically on lysine 18. We had previously reported that *L. monocytogenes* imposes histone modifications (3), but here we demonstrate that a histone modifier is essential for infection.

Materials and Methods

Antibodies

We used the rabbit polyclonal antibodies anti-acetyl-histone H3 K9 (Cell Signaling, catalog no. 9671), anti-acetyl-histone H3 K14 (Cell Signaling, 4318), anti-acetyl-histone H3K18 (Cell Signaling, 9675), anti-histone H3 (Cell Signaling, 9715), anti-histone H4 (AbCam, ab10158), anti-acetyl-histone H4 K16 (Millipore, 06-762), anti-trimethyl-histone H3 K9 (Upstate, 07-442), and anti-SIRT2 (Thermo Scientific, PA3-200). We used the mouse monoclonal antibodies anti-HP1-1 α (Euromedex, 2HP-1H5-AS), anti- α -tubulin (Sigma, T6074), and anti-actin (Sigma, A5441).

Cell Culture, Infections, and Inhibitors

The bacterial strains that we used in this study are indicated in table S1. *L. monocytogenes* strains were grown in brain-heart infusion medium (Difco, Detroit, Michigan) at 37°C until the optical density at 600 nm = 1. When required, antibiotics were added (chloramphenicol, 7 or 35 μ g/ml; erythromycin, 5 μ g/ml).

HeLa (ATCC CCL-2) and CaCO₂ (ATCC HTB-37) cells were cultured in minimum essential medium (MEM) plus GlutaMAX (GIBCO) supplemented with 1 mM sodium pyruvate (GIBCO), 0.1 mM nonessential amino acid solution (GIBCO), and 10% (HeLa) or 20% (CaCO₂) fetal calf serum (FCS). RAW 264.7 cells (ATCC TIB-71) were cultured in Dulbecco's minimum essential medium plus GlutaMAX (GIBCO) supplemented with 2 mM glutamine (GIBCO), 1 mM sodium pyruvate, and 10% FCS.

HeLa and CaCO₂ were grown to semiconfluence, at which point they were serum-starved (serum low medium: MEM plus GlutaMAX, 1 mM Na²⁺ pyruvate, 0.1 mM nonessential amino acid solution, 0.25% FCS) for 24 hours before use in experiments. Exponential phase bacteria were washed twice in the above-mentioned serum-low medium and added to cells at a multiplicity of infection of 50:1 (HeLa and CaCO₂). After 1 hour of infection, cells were washed with serum-low medium, and 10 μ g/ml gentamycin was added. Infections were carried out for 5 hours unless otherwise indicated.

InIB was isolated as per Ireton *et al.* (51). Cells were treated with purified phosphate-buffered saline [PBS, InIB (10 ng/ml), HGF (10 ng/ml, Sigma, H5791)] or EGF (100 nM, Sigma, E9644). Polystyrene carboxyl P(S/V-COOH/1) 1.1- μ m beads (Bang Laboratories, via biovalley.fr, PC04N-7740-11) were coated with InIB, as per protocol (Bang Laboratories, Beads Above the Rest, TechNote 205, Covalent Coupling).

For experiments involving pharmacological inhibitors, cells were pretreated for 2 hours before infection with TSA (5 μ M, Sigma, T8552),

NIC (5 μ M, Sigma, N0636), CTCC (5 μ M, Enzo Life Sciences, ALX-270-437), and AGK2 (5 μ M, Enzo Life Sciences, ALX-270-484) or for 30 min with PBS, dimethyl sulfoxide, wortmannin (100 nM, Sigma, W1628), genistein (10 μ M, Sigma, G6776), and HIMO (10 μ M, Alexis Biochemicals, ALX-270-292).

Cloning

For overexpression of WT SIRT2, we used a SIRT2-GFP construct (pEGFP-c1 backbone; EGFP, enhanced green fluorescent protein), which was a gift from B. North (24). siRNA-insensitive SIRT2 and SIRT2 N168A were constructed by PCR amplification of the SIRT2-GFP construct with primer pairs: (i) SIRT2 fwd and SIRT2 05 rev, (ii) SIRT2 05 fwd and SIRT2 rev, and (iii) SIRT2 fwd and SIRT2 rev (see tables S3 and S4 for primer sequences), followed by insertion at the EcoRI site of the pEGFP-c1 vector.

Transfections

DharmaFECT (Dharmacon) transfection was used to introduce RNA interference knockdown SIRT1 (Thermo Scientific, siGENOME SMARTpool M-003540_01_0005), SIRT2 (Thermo Scientific, siGENOME SMARTpool M-004826-02-0005, or siRNA fragment D-004826-05), or control scramble siRNA (On-TARGETplus SMARTpool). Cells were assayed 72 hours after siRNA transfection.

SIRT2-FLAG or SIRT2N168A-FLAG were rendered sensitive to siRNA by the addition of three point mutations by PCR amplification using primers SIRT2 fwd with SIRT2 rev 05 and SIRT2 fwd 05 with SIRT2 rev (see tables S3 and S4 for primer list).

Immunoblotting and Cell Fractionation

Total cell lysates were harvested by removing growth medium and adding lysis buffer [1M Tris HCl (pH 6.8 at 25°C), 10% SDS, 50% glycerol, 0.05% bromophenol blue, 10% β -mercaptoethanol]. Spleen and liver samples were collected as described in (52). Samples were boiled for 5 to 10 min, sonicated for 5 s, and loaded on a 15% acrylamide gel. A semidry transfer was conducted for 1 hour, at 32 mA per transfer, followed by blocking of the Hybond P-polyvinylidene difluoride membranes (GE Healthcare) in tris-buffered saline-Tween [Tris 50 mM (pH 8), NaCl 15mM, 0.1% Tween] supplemented with 10% milk. Transferred membranes were incubated with previously mentioned primary antibodies for 2 hours at 25°C or at 4°C. Membranes were washed and incubated with horseradish peroxidase-conjugated goat α -rabbit or α -mouse antibodies (Bioss Laboratories). Quantification of Western blots was performed using the G:box-ichemi machine (SynGene).

Cell fractionation was conducted as follows: Cells were resuspended in buffer A [20 mM HEPES (pH 7.0), 0.15 mM EDTA, 0.15 mM EGTA, 10 mM KCl]. 1% NP40 was added, followed by SR buffer [50 mM HEPES (pH 7.0), 0.25 EDTA, 10 mM KCl, 70% (m/v) saccharose].

Samples were centrifuged for 5 min at 2000 g. The supernatant was isolated as the cytosolic fraction and recentrifuged for 20 min at 20,000 g to eliminate cell nuclear debris. The pellet was washed in buffer B [10 mM HEPES (pH 8.0), 0.1 mM EDTA, 100 mM NaCl, 25% (v/v) glycerol] and centrifuged for 5 min at 2000 g. Buffers A, B, and SR were supplemented with 0.15 mM spermidine, 0.15 mM spermine, 1 mM dithiothreitol, and 1× Complete (Roche). The washed pellet was resuspended in sucrose buffer [20 mM Tris (pH 7.65), 60 mM NaCl, 15 mM KCl, 0.34 M sucrose, 0.15 mM spermine, 0.15 mM spermidine], followed by the addition of a high-salt buffer [20 mM Tris (pH 7.65), 0.2 mM EDTA, 25% glycerol, 900 mM NaCl, 1.5 mM MgCl₂] to obtain a final salt concentration of 250 mM. Samples were incubated for 25 min and centrifuged for 10 min at 10,000 revolutions per minute (rpm). The supernatant was isolated as the nuclear soluble fraction from the pellet, which represents chromatin and nuclear insoluble material. The pellet was resuspended in sucrose buffer + MNase (0.0025 units/ul) and 1 mM CaCl₂ and was incubated at 37°C for 10 min. 4 mM EDTA was added, and samples were sonicated using the Bioruptor (Diagenode) for 7.5 min (15 s on and 1 min off) and centrifuged for 15 min at 13,000 rpm. The supernatants represent a soluble chromatin fraction.

Immunofluorescence and FACS Analysis

Cells were grown on glass cover slides. After infection, cells were fixed in 4% paraformaldehyde and permeabilized in 0.3% triton for 15 min. Immunostaining was performed with an anti-SIRT2 antibody (Thermo Scientific, PA3-200) in 1% BSA+ 0.1% Tween 100. Infections for immunofluorescence and FACS were carried out with GFP-expressing *L. monocytogenes*. Infected cells were monitored with a FACScalibur (BD Bioscience), and analysis was done with FlowJo software (www.flowjo.com/).

Microarray Analysis

Total mRNA from uninfected and infected cells, pretreated or untreated with AGK2 (5 μM for 2 hours), was extracted and purified as per an RNeasy kit (Qiagen, Valencia, California). Quality assessment and normalization of the arrays was performed with the tools available in the Expression Console v1.1 (Affymetrix) and Bioconductor packages. Total RNA (200 ng) was reverse-transcribed and amplified per the manufacturer's protocols using the Applause WTA Amp-Plus System (Nugen Technologies, 5510-24) and was fragmented and biotin-labeled using the Encore Biotin Module (Nugen Technologies, 4200-12). Gene expression was determined by hybridization of the labeled template to *HuGene 1.0 ST* microarrays (Affymetrix, Santa Clara, California). Hybridization cocktail and posthybridization processing were performed according to the "Target Preparation for Affymetrix GeneChip Eukaryotic Array Analysis" protocol found in

the appendix of the Nugen protocol of the fragmentation kit. Arrays were hybridized for 18 hours, washed using fluidics protocol FS450_0007 on a GeneChip Fluidic Station 450 (Affymetrix), and scanned with an Affymetrix Genechip Scanner 3000, generating CEL files for each array. Three biological replicates were run for each condition.

Gene-level expression values were derived from the CEL file probe-level hybridization intensities using the model-based robust multichip average algorithm (RMA) (53). RMA performs normalization, background correction, and data summarization. An analysis was performed using the Limma *t* test (54), and a *P* value threshold of *P* < 0.05 was used as the criterion for expression. The estimated false discovery rate (FDR) of this analysis was calculated with the Benjamini-Hochberg approach (55) to correct for multiple comparisons.

qRT-PCR

Total mRNA was extracted using the RNeasy kit (Qiagen). Reverse transcription was performed with the iScript cDNA Synthesis kit (BioRad). Qualitative PCR (qPCR) was done using the SsoFast EvaGreen Supermix (BioRad) and run on a MyIQ device (BioRad). Data were analyzed by the $\Delta\Delta C_t$ method.

Chromatin Immunoprecipitation

Preparation of ChIP samples was adapted from Lebreton *et al.* (56). Chromatin inputs corresponded to 1.8×10^5 cells for each individual ChIP assay. All buffers were supplemented with Complete EDTA-free protease inhibitor cocktail tablets (Roche). Formaldehyde-fixed cells were washed in PBS and lysed in 10 mM Tris (pH 8), 10 mM EDTA, 0.5 mM EGTA, 0.25% Triton X-100 for 5 min on ice. The nuclear pellets were recovered by brief centrifugation at 3000 × g, and the soluble nuclear fraction was extracted with 250 mM NaCl, 50 mM Tris (pH 8), 1 mM EDTA, 0.5 mM EGTA for 30 min on ice. After brief centrifugation at 16,000 × g, chromatin pellets were resuspended in 10 mM Tris (pH 8), 1 mM EDTA, 0.5 mM EGTA, 0.5% SDS and then sonicated with a Bioruptor (Diagenode) to shear chromatin to a final size of 150 to 600 base pairs. Extracts were quantified by an absorbance of 260 nm, and material quantities were adjusted accordingly. Samples were then diluted to obtain the following immunoprecipitation (IP) buffer composition: 150 mM NaCl, 10 mM Tris (pH 8), 0.1% SDS, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM EDTA, 0.5 mM EGTA. IP was carried out in the on setting at 4°C with antibodies to SIRT2, H3, H3K18 Ac, H4, and H4K16 Ac. Immunocomplexes were recovered with Dynabeads Protein G (Invitrogen), added for 90 min at 4°C, and then washed five times in a succession of isotonic and saline buffers as described in (57). After a final wash in 10 mM Tris pH8, 1 mM EDTA, 0.01% Igepal, bound material was eluted by the addition of water containing 10% Chelex (Bio-Rad), followed

by boiling for 10 min to reverse the cross-link. Samples were then incubated with proteinase K (100 μg/ml) for 30 min at 55°C with some shaking and then boiled for another 10 min. Finally, the ChIP DNA fraction was separated from beads and the Chelex matrix by centrifugation. Recovered supernatants were quantified by qualitative real-time PCR (qRT-PCR) using the $\Delta\Delta C_t$ method. Results for samples immunoprecipitated with Ach3K18 were normalized to samples immunoprecipitated with H3. The sequences for the primers used are given in tables S3 and S4.

Sirt2 Mice

Sirt2^{tm1a(EUCOMM)Wsi} mice were obtained from the Sanger Center. For details, see www.informatics.jax.org/javawi2/servlet/WIFetch?page=alleleDetail&key=606707. Infections were performed by intravenous injection of 10⁵ bacteria per animal. Experiments were performed according to the Institut Pasteur guidelines for animal experimentation.

Immunoprecipitation

Immunoprecipitation of SIRT2-FLAG was performed with M2-FLAG affinity gel (Sigma, A2220), according to the manufacturer's protocol. Elution was performed in 0.1 M glycine HCl, pH 3.5.

SDS-PAGE and LC-MS/MS Analysis

Proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 4 to 15% polyacrylamide gel (Bio-Rad) and stained by colloidal coomassie blue (Invitrogen). For every sample, the gel lane was cut into five consecutive gel slices that were washed with H₂O, incubated for 15 min with water/acetonitrile (1/1, v/v), and incubated for 15 min with 100% acetonitrile before drying completely in a vacuum concentrator. Sequencing-grade trypsin (0.25 μg, Promega) in 50 mM ammonium bicarbonate in water/acetonitrile (9/1, v/v) was added to the dried gel slices, and proteins were digested overnight at 37°C. Peptides eluted from every gel slice were dried completely in a vacuum concentrator and redissolved in 15 μl solvent A [0.1% formic acid in water/acetonitrile (98/2, v/v)], 5 μl of which were used for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis on an Ultimate 3000 high-performance LC system (Dionex) in line connected to an LTQ Orbitrap Velos mass spectrometer (Thermo Electron). Trapping was performed at 10 μl/min for 4 min in solvent A on a PepMap C18 column [0.3-mm inner diameter by 5 mm (Dionex)]; after back-flushing from the trapping column, the sample was loaded on a reverse-phase column (made in house, 75-μm inner diameter by 150 mm, 3-μm beads C18 Reprosil-HD, Dr. Maisch, Entringen, Germany). Peptides were eluted by a linear increase from 2 to 55% solvent B [0.08% formic acid in water/acetonitrile (2/8, v/v)] over 30 min at a constant flow rate of 300 nL/min.

The mass spectrometer was operated in data-dependent mode, automatically switching between MS and MS/MS acquisition for the 10 most

abundant ion peaks per MS spectrum. Full-scan MS spectra [mass-to-charge ratio (m/z) of 300 to 2000] were acquired at a resolution of 60,000 in the orbitrap analyzer after accumulation to a target value of 1,000,000. The 10 most intense ions above a threshold value of 5000 were isolated for fragmentation by collision-induced dissociation at a normalized collision energy of 35% in the linear ion trap (LTQ) after filling the trap at a target value of 5000 for a maximum of 50 ms. From the MS/MS data in each LC run, Mascot generic files were created using the Mascot Distiller software (version 2.4.3.3, Matrix Science). When generating peak lists, grouping of spectra was performed with m/z 0.005 tolerance on the precursor ion, a maximum intermediate retention time of 30 s, and a maximum intermediate scan count of five. A peak list was only generated when the MS/MS spectrum contained more than 10 peaks, no deisotoping was performed, and the relative signal-to-noise limit was set at two.

Generated peak lists were then searched with Mascot using the Mascot Daemon interface (version 2.3.0, Matrix Science) against the human proteins in the Swiss-Prot database (database release version of 7 July 2012 containing 20,235 human protein sequences). Variable modifications were set to oxidation of methionine residues; pyroglutamate formation of N-terminal glutamine residues; acetylation of peptide N termini and lysine residues; di-glycine modification of lysine residues; and phosphorylation of serine, threonine, and tyrosine residues. Mass tolerance of the precursor ions was set to 10 parts per million; mass tolerance of the fragment ions was set to 0.5 daltons. The peptide charge was set to 1⁺, 2⁺, or 3⁺, and one missed tryptic cleavage site was allowed. Also, the C13 setting of Mascot was set to 1. Only peptides that were ranked first and scored above the threshold score set at 99% confidence were withheld. For processing of all MS data, the ms_lims software platform was used [PubMed identifier (PMID) 20058248]. In addition, the C13 setting of Mascot was set to 1. Only peptides that were ranked first and scored above the threshold score set at 99% confidence were withheld. In total, 18,834 fragmentation spectra were identified (with a FDR of 0.23%) (PMID 18067246). For processing of all MS data, the ms_lims software platform was used (PMID 20058248).

Histone Purification

Histones were purified according to protocol published in (58) using trichloroacetic acid precipitation.

SDS-PAGE, in Gel Propionylation and LC-MS/MS Analysis

Deacetylation of H3K18 by differential MS was performed using an adapted version of the protocol of Garcia *et al.* (59). Briefly, equal amounts (100 μ g) of purified histones from infected and uninfected samples were separated by SDS-PAGE on a 16.5% Tris-tricine gel (Biorad) and

stained by coomassie blue. Protein bands corresponding to histone H3 were cut and modified by in-gel differential propionylation (60, 61). Histone H3 bands were cut and washed with H₂O, incubated for 15 min with water/acetonitrile (1/1, v/v), and incubated for 15 min with 100% acetonitrile before being dried completely in a vacuum concentrator. Isotopically light (¹²C₃)– or heavy (¹³C₃)–labeled *N*-hydroxysuccinimide propionate (1 mg) (provided by K. Gevaert and B. Rutters, Ghent University, Belgium) was then added in 150 μ l 100 mM sodium phosphate (pH 8.0) to the gel pieces of the EGD infected and uninfected samples, respectively, and both samples were incubated for 1 hour at 30°C. To ensure quantitative labeling, this step was repeated after washing and drying the gel samples again, as described above. After this second propionylation step, gel pieces were washed twice with 200 μ l 50 mM ammonium bicarbonate (pH 8.0) for 10 min to remove and quench excess reagents. Water-hydroxylamine (2 μ l, 1/1, v/v, Sigma) was then added to the final wash solution, and samples were incubated for 20 min at room temperature to revert possible O-acylation of Ser, Thr, or Tyr residues. To ensure complete oxidation of methionine residues, gel samples were washed with 200 μ l 0.1% trifluoroacetic acid (TFA) for 10 min at 30°C and then incubated with 200 μ l 0.5% hydrogen peroxide in 0.1% TFA for 30 min at 30°C. Gel samples were washed for 10 min with 200 μ l acetonitrile and dried completely in a vacuum concentrator. Sequencing-grade trypsin (0.15 μ g, Promega) in 50 mM ammonium bicarbonate in water/acetonitrile (9/1, v/v) was added to the dried gel slices, and proteins were digested overnight at 37°C. Equal amounts of peptides eluted from both gel samples were mixed, dried completely in a vacuum concentrator, and re-dissolved in 40 μ l solvent A [0.1% formic acid in water/acetonitrile (98/2, v/v)], 5 μ l of which was injected for LC-MS/MS analysis on a LTQ Orbitrap Velos mass spectrometer (Thermo Electron). LC-MS/MS analysis, peak list generation, and Mascot searches occurred with only small adjustments to the search settings: Oxidation of methionines residues was set as fixed modification (+15.994915 daltons), whereas acetylation (+42.010565 daltons) and light (+56.026215 daltons) and heavy (+59.036279 daltons) propionylation of lysine residues were set as variable modifications. Also, a maximum of two missed tryptic cleavage sites was allowed.

A recent paper has reported that the bacterium *Legionella* secretes a protein named RomA that trimethylates K14 of histone H3, leading to repression of a number of host genes (63).

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 62. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Supplementary Materials
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 Figs. S1 to S10
 Tables S1 to S5

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The Evolution of Social Monogamy in Mammals

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The evolution of social monogamy has intrigued biologists for over a century. Here, we show that the ancestral condition for all mammalian groups is of solitary individuals and that social monogamy is derived almost exclusively from this social system. The evolution of social monogamy does not appear to have been associated with a high risk of male infanticide, and paternal care is a consequence rather than a cause of social monogamy. Social monogamy has evolved in nonhuman mammals where breeding females are intolerant of each other and female density is low, suggesting that it represents a mating strategy that has developed where males are unable to defend access to multiple females.

Despite extensive interest in the evolution of monogamy stimulated by its prevalence in humans (1–3), the distribution of social monogamy in nonhuman mammals continues to puzzle evolutionary biologists (4). In contrast to birds, social monogamy in mammals is usually associated with genetic monogamy, and the incidence of extra-pair mating is generally low in socially monogamous societies (5). There are two main explanations for its existence. One suggests that it is a consequence of selection for some form of paternal care, such as contributions to carrying or provisioning young or their protection from infanticide by competing males (6). Alternatively, social monogamy may represent a mate guarding strategy and may have evolved where males were unable to defend access to more than one female (7, 8), either because of mutual intolerance between breeding females (9, 10) or because large female home ranges prevent effective defense by males of territories covering the ranges of more than one female (11).

A recent comparative analysis of primate social systems (12) using a Bayesian approach identified six transitions to social monogamy in primates and concluded that social monogamy is derived from an ancestral condition where both sexes are social and live in unstable groups, supporting the suggestion that its evolution may be associated with the risk of male infanticide. However, this seems unlikely to provide a general explanation for the evolution of social monogamy in mammals because groups of breeding females occur much less frequently in other taxonomic groups. We used data for more than 2500 mammals to identify 61 independent evolutionary transitions to social monogamy in mammals, assess the characteristics of the species in which transitions occurred, and test the

predictions of alternative explanations of the evolution of social monogamy.

We classified the social systems of all nonhuman mammalian species for which information was available ($n = 2545$) as either solitary (breeding females forage independently in individual home ranges and encounter males only during mating), socially monogamous (a single breeding female and a single breeding male share a common range or territory and associate with each other for more than one breeding season, with or without nonbreeding offspring), or group living (several breeding females share a common range and forage or sleep together). Group-living species (which typically have polygynous or polygynandrous mating systems) include those where groups of breeding females are unstable, as in the case of ungulate herds or the roosting groups of some bats, as well as species where several breeding females associate with each other in stable groups for more than one breeding season, whether or not they always forage together (see supplementary materials and supplementary data). Although in some nonhuman mammalian species smaller social groups occasionally merge to form larger unstable groups (as in elephants and gelada baboons), associations of socially monogamous pairs, which are common in birds, have not been reported except possibly in the mara, *Dolichotis patagonum* (13). Species were classified as showing paternal care if males regularly contribute to feeding or carrying offspring (2, 14). After reconstructing the most parsimonious sequence of transitions across a recently derived mammalian supertree (15), all inferences were confirmed by using likelihood-based reconstruction approaches (16, 17). We first tested for associations between the distribution of social monogamy and several social and ecological traits by using nonparametric tests, phylogenetic independent contrasts (18), and regression models that account for phylogenetic relatedness (19–21). Next, we assessed the importance of any associated factors in predicting transitions to social monogamy by com-

paring inference models in BayesTraits' Discrete and Multistate (17, 22).

The Distribution of Social Monogamy

Of the 2545 mammalian species whose social systems could be classified, breeding females were classified as solitary in 1741 species (68%), socially monogamous in 229 species (9%), and living in social groups in 575 species (23%). The proportion of socially monogamous species in our sample is slightly higher than frequently reported earlier estimates [3% (1)] but is still an order of magnitude lower than in birds, where 90% of species are considered to be socially monogamous (23). Social monogamy occurs more frequently in some mammalian orders, such as Primates (106 of 361 species, 29%) and Carnivora (33 of 201 species, 16%), and is uncommon in others, such as Artiodactyla (6 of 187 species, 3%), and absent in a few, including Cetacea (table S1).

Transitions to Social Monogamy

Our phylogenetic reconstruction shows that, in the common ancestor of all mammalian species, females were solitary and males occupied ranges or territories overlapping several females. All approaches to reconstructing evolutionary sequences support this inference for the 2288 species included in the updated mammalian supertree, and the likelihood that the common ancestor was solitary is 0.99 for all approaches. Solitary living appears to have been the ancestral condition for the ancestors of all mammalian orders, with the possible exception of elephant shrews (Macroscelidea) and hyraxes (Hyracoidea). Closely related species generally have the same social system, and female sociality has a strong phylogenetic signal: Maximum likelihood estimate of Pagel's lambda was 0.93 for solitary living, 0.92 for social monogamy, and 0.86 for group living; all lambda estimates were significantly different from 0 (no phylogenetic signal) on the basis of likelihood ratio tests. Similarly, the phylogenetic signal for all three social systems combined was significantly different from a chance distribution of sociality across species [signal of 0.20, Z-score from randomization = -10.99, $P = 0.001$; see (21)].

Parsimonious reconstructions suggest that 61 independent transitions to social monogamy from solitary ancestors are necessary to explain the distribution of social monogamy among current species. In all but one case, socially monogamous species in our data set appear to have been derived from an ancestor where females were solitary and lived in individual home ranges and males ranged independently: The only potential transition to social monogamy from an ancestor that is likely to have lived in groups occurs in the primate genus *Eulemur*. The results of BayesTraits' Discrete and Multistate analyses performed for each order in which socially monogamous species occur confirmed that social monogamy is almost exclusively derived from ancestors where females are solitary. The most likely models suggest

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that no transitions to social monogamy from group living occurred except in the one instance in the primates. Models in which transition rates to social monogamy were forced to occur equally from group-living ancestors and solitary ancestors performed significantly worse than models in which all socially monogamous species are derived from a solitary ancestor [likelihood ratio test (lrt) all $P < 0.005$; table S2]. Group-living sister taxa of socially monogamous species occur in some groups (e.g., banded mongooses, *Mungos mungo*; Goeldii's monkey, *Callimico goeldii*; sifakas, *Propithecus* spp.) and probably represent secondary transitions to group living from socially monogamous ancestors.

Social Monogamy and Male Care

Although it is often difficult to exclude the possibility of any form of male contribution to the care of young, detailed field studies have found no evidence of any form of male contribution to care in 94 of 229 (41%) socially monogamous species. For example, in dik-dik, where males are both genetically and socially monogamous and are closely associated with their mates, they provide no contributions to guarding, carrying, feeding, or teaching young or to any other obvious form of paternal care (24). The distribution of paternal care in contemporary socially monogamous species is closely associated with the form and distribution of maternal care: Where females carry and/or pro-

vision offspring, males commonly contribute to the same activities. Regular provisioning or carrying of young by males has been recorded in 135 (59%) of the 229 socially monogamous mammals, whereas it is found only in three non-monogamous species, two of which appear to be derived from a socially monogamous ancestor [*M. mungo* (25) and *C. goeldii* (26); the third species is *Hapalemur griseus* (27)].

Comparisons suggest that paternal care probably contributes to the fitness of both sexes: Females in socially monogamous species with biparental care produce more litters per year (median = 2, range from 0.9 to 9, $n = 48$ species) than in socially monogamous species without biparental care [median = 1, range from 0.2 to 7, $n = 37$ species; analysis of variance (ANOVA) $F = 4.43$, $P = 0.04$, phylogenetic generalized least squares (phy): $\lambda = 0.92$, $t = -2.6$, $P = 0.01$] or in solitary species (median = 1.1, range from 0.2 to 7, $n = 242$ species; $F = 7.56$, $P = 0.006$, phy: $\lambda = 0.97$, $t = 2.1$, $P = 0.03$). Increases in the reproductive rate of females probably have benefits to males, who sire offspring in more breeding cycles in socially monogamous species with paternal care (median = 6 breeding seasons, range from 4.5 to 8 breeding seasons, $n = 11$ species) than in socially monogamous species where males do not provide care (median = 3 breeding seasons, range from 2 to 8 breeding seasons, $n = 8$ species; $F = 4.98$, $P = 0.04$; phy: $\lambda = 0.78$, $t = 2.0$,

$P = 0.06$), even though there are no differences in male tenure length (with paternal care median = 47 months, without median = 45 months; $F = 2.10$, $P = 0.17$, phy: $\lambda = 0.53$, $t = -1.1$, $P = 0.31$).

Although paternal care and social monogamy are associated, an analysis of transitions suggests that male care is probably a consequence rather than a cause of the evolution of social monogamy. About half of all independent transitions to paternal care have occurred in instances where social monogamy was already established, whereas the evolution of paternal care occurred on the same branch as a transition to social monogamy in the other cases. Inferences from BayesTraits' models indicate that paternal care is a secondary adaptation, because transitions to social monogamy are inferred to occur first on branches where both traits evolved separately (lrt $P = 0.002$; table S2).

Social Monogamy and Male Infanticide

An alternative suggestion is that social monogamy allows males to protect their offspring from attacks by infanticidal competitors and has evolved for this reason (28). However, the available evidence suggests that male infanticide is unlikely to be the principal mechanism for the evolution of social monogamy in mammals. Male infanticide is typically found in species where the duration of lactation exceeds the duration of gestation (6, 28): This is the case in few socially monogamous species (20 of 75 species, 27%) compared with species where females are solitary [148 of 335 species, 44%; Wilcoxon $W = 11733$, $P = 0.34$; phylogenetic independent contrasts (pic) $t = -1.63$, $P = 0.10$], and BayesTraits' models also provide no evidence of an association between the evolution of social monogamy and lactation durations that exceed gestation (lrt $P > 0.40$, table S2). Although the prevalence of male infanticide is lower among socially monogamous species (4 of 47 species, 9%) than among solitary species (24 of 88 species, 27%; $W = 1542.5$, $P = 0.01$), this difference does not appear to be a consequence of a direct association between social monogamy and male infanticide, because an analysis of phylogenetic independent contrasts ($t = -0.402$, $P = 0.69$) and BayesTraits' models suggest an independent evolution of the two traits (lrt $P > 0.90$, table S2).

Social Monogamy and the Ecological Defensibility of Females

The main alternative explanation of the distribution of social monogamy in mammals is that it has evolved where females are solitary and males are unable to defend access to more than one female at a time (7). Evidence that socially monogamous species are derived from ancestors where females are solitary (see above) supports this suggestion. Moreover, unlike previous analyses (2, 4), our data show that socially monogamous mammals live at significantly lower densities (median of 15 individuals per square kilometer, $n = 89$ species) than solitary species [median of 156

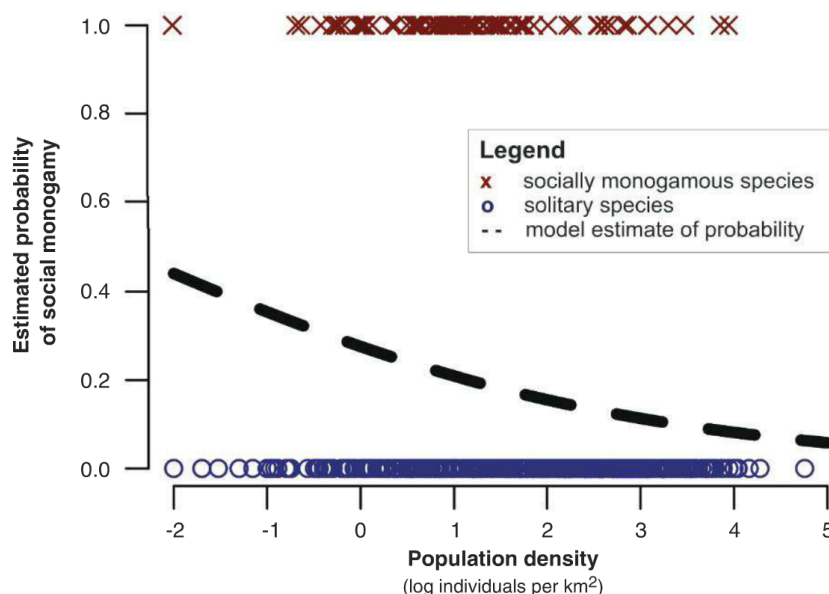


Fig. 1. Fitted values of the probability that a species is socially monogamous given a population density obtained by a binomial GLM (dashed line). The blue dots are the observed values for solitary species ($n = 411$), the red crosses the observed values for socially monogamous species ($n = 89$, 18% of all species), and values can overlap (e.g., there are four socially monogamous species with a log population density of -2). Population density (logarithm of the number of individuals per km^2) has a substantial influence on the probability of that a species is socially monogamous or solitary. At the highest population densities, there is only a 6% probability that a species will be socially monogamous, whereas the probability rises to 44% at the lowest population densities. Several of the socially monogamous species showing high population densities are cooperative breeders, where many of the adult individuals do not breed.

individuals per square kilometer, $n = 411$ species; $W = 10746.5$, $P < 0.001$; phylogenetically controlled binomial generalized linear model (GLM) in MCMCglmm (pMCMC) $P = 0.007$] (Fig. 1). Socially monogamous species have, on average, higher individual body mass (median = 873 g) compared with solitary species (median = 308 g; $W = 40733$, $P = 0.001$; pMCMC = 0.34), which may contribute to their low density. However, the residuals of a phylogenetically controlled regression of population density on body mass are significantly lower for socially monogamous species than for solitary species ($W = 10421$, $P < 0.001$;

pMCMC < 0.001), indicating that size differences alone do not account for the low density of socially monogamous species.

Despite the association between social monogamy and low population density, there is no significant difference in female home-range size between socially monogamous (median = 0.21 square kilometers, $n = 71$ species) and solitary (median = 0.53 square kilometers, $n = 185$ species; $W = 5553$, $P = 0.06$; pMCMC = 0.11) species, even when differences in body mass are controlled for ($W = 6100$, $P = 0.70$; pMCMC = 0.08). This suggests that there may be greater

overlap of home ranges between females in solitary species than in socially monogamous ones, and comparative data for primates (the only taxonomic group for which comparative data are available) support this conclusion: In a sample of 26 socially monogamous primates, home ranges overlap on average by 21% (median = 17%), whereas the ranges of females overlap on average by 49% (median = 58%, $n = 5$ species; $F = 7.08$, $P = 0.01$; phy: lambda = 0.0, $t = -2.4$, $P = 0.02$) in species where females are solitary.

The high incidence of social monogamy in Primates and Carnivora compared with more herbiv-

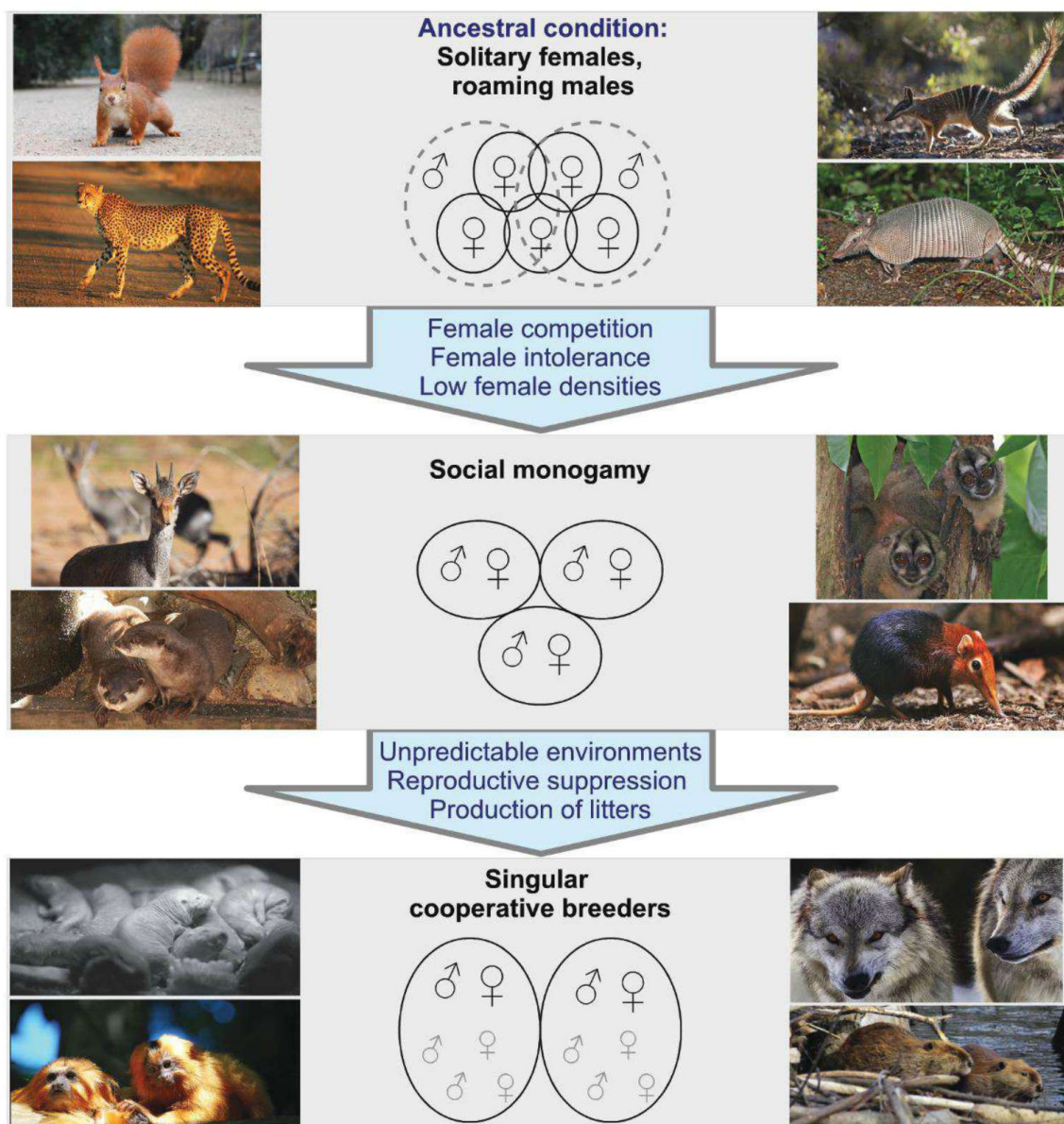


Fig. 2. Evolutionary pathway to monogamy and singular cooperative breeding in mammals. In mammals, social monogamy derives from ancestral social systems in which females are solitary and male ranges overlap those of several females. Social monogamy appears to have evolved in species where females rely on high-quality, low-density diets; breeding females are intolerant of each other; and female density is low, preventing breeding males from guarding more than one breeding female. In some monogamous lineages where females

are polytocous and habitats are unpredictable, systems where one female monopolizes breeding and her young are raised by other group members who are typically close relatives that have not yet left their natal group have evolved (29). [Photo credits: red squirrel (33), numbat (34), cheetah (35), armadillo (36), dik-diks (37), night monkeys (38), small-clawed otters (39), elephant shrew (40), naked mole rats (41), wolves (42), golden lion tamarins (43), beavers (44). All photos made available under Creative Commons attribution licenses.]

orous orders (including Rodentia and Artiodactyla) suggests that the evolution of low range-overlap in females and social monogamy may be a consequence of a reliance on resources of high nutritional quality but low abundance. Comparisons show that a similar association between social monogamy and low-density resources occurs within orders. For example, in 91% (81 of 89) of socially monogamous primates, fruit constitute the main part of the diet, whereas fruit is the single most important food for only 28% (13 of 46) of solitary primate species ($W = 762.5$, $P < 0.001$; pic $t = 3.12$, $P = 0.002$). In contrast, foods of low nutritional value (gum, bark, fungi) are included in the diet of significantly more solitary (43 of 46, 93%) than socially monogamous primate species (35 of 89, 39%; $W = 3155.5$, $P < 0.001$; pic $t = -4.18$, $P < 0.001$).

Analyses of patterns of sexual dimorphism also suggest that competition between females may be more intense in socially monogamous species than in solitary. Although males are heavier than females in 134 of 170 species (79%) where females are solitary, male-biased sexual dimorphism is found in only 21 of 44 socially monogamous species (48%; $W = 2736.5$, $P < 0.001$; pic $t = 1.53$, $P = 0.13$). This difference does not appear to be a consequence of a reduction in dimorphism after the transition to social monogamy, for the sequence of transitions (as inferred by the most likely BayesTraits' models) suggests that social monogamy only evolved in species in which females are at least as large as males (1rt $P < 0.05$; table S2) and that in some socially monogamous species changes in evolutionary conditions appear to have led to subsequent increases in sexual dimorphism, preceding the loss of social monogamy.

Discussion

Like previous analyses (2, 12), our results suggest that the evolution of social monogamy has been restricted to particular ancestral states. However, our conclusion that social monogamy is derived from an ancestral state in which females are solitary and male ranges overlap those of several females contrasts with recent suggestions that, in primates, it is derived from ancestors in which females and males live in unstable groups (12). This difference is unlikely to be a consequence of contrasts between primates and other mammals, for five of the six transitions to social monogamy among primates in our data set were also from ancestors where females were solitary. Instead, it is likely to be a consequence of a contrast in the classification of social systems: Shultz *et al.* classify socially monogamous species that are accompanied by nonbreeding offspring as group living and do not distinguish between social systems of this kind and plural breeders, where groups include several breeding females. As a result, some species that we classify as socially monogamous were classified by Shultz *et al.* as group living. This difference in classification highlights the extent to

which the way in which social systems are classified can influence the interpretation of species differences.

The association between social monogamy and low population density also differs from previous analyses, which found no significant difference in population density between socially monogamous species and those where females live in separate home ranges (2, 4). In this case, it seems likely that the contrast is a result of differences in sample size between our analyses and previous analyses, where sample size was less than 90 species (2, 4). Our larger sample size also allowed us to assess whether changes in population density preceded transitions to social monogamy, whereas comparing average population density between solitary and socially monogamous species may fail to detect a difference because changes to low population density in social species might not necessarily lead to the evolution of social monogamy (14).

Our results suggest that social monogamy evolved in mammals where feeding competition between females was intense, breeding females were intolerant of each other, and population density was low (Fig. 2). Under these conditions, guarding individual females may represent the most efficient breeding strategy for males (7). The evolution of paternal care appears to have succeeded the evolution of social monogamy, suggesting that it is unlikely to be a precondition for its evolution. Transitions to singular cooperative breeding occurred in a small number of socially monogamous species (29), and occasionally plural breeding by several females whose offspring are raised by all group members evolved from such an ancestor [e.g., banded mongooses (25)]. This suggests that there are at least two independent routes to female sociality in mammals.

Because all the African apes are polygynous and group living, it is likely that the common ancestor of hominids was also polygynous, and this is supported by evidence of substantial sexual size dimorphism in early hominids (30), as well as by sex differences in rates of aging in modern humans (31). It has been suggested that the evolution of human monogamy could have been a consequence of the need for extended paternal investment (3). Alternatively, the rarity of transitions to social monogamy from group-living, polygynous species in nonhuman mammals could suggest that the shift to monogamy in humans may be instead the result of a change in dietary patterns that reduced female density and limited the potential for males to guard more than one female (32).

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Supplementary Materials

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REPORTS

Hierarchical Porous Polymer Scaffolds from Block Copolymers

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Hierarchical porous polymer materials are of increasing importance because of their potential application in catalysis, separation technology, or bioengineering. Examples for their synthesis exist, but there is a need for a facile yet versatile conceptual approach to such hierarchical scaffolds and quantitative characterization of their nonperiodic pore systems. Here, we introduce a synthesis method combining well-established concepts of macroscale spinodal decomposition and nanoscale block copolymer self-assembly with porosity formation on both length scales via rinsing with protic solvents. We used scanning electron microscopy, small-angle x-ray scattering, transmission electron tomography, and nanoscale x-ray computed tomography for quantitative pore-structure characterization. The method was demonstrated for AB- and ABC-type block copolymers, and resulting materials were used as scaffolds for calcite crystal growth.

Hierarchically porous scaffolds provide synergies between mechanical properties, transport properties, and enhanced surface area (1). Integrating mesoscale (2 to 50 nm) porosity with three-dimensional (3D) continuous macropores (>50 nm) is of particular importance because it combines high specific surface area with high flux and pore accessibility desired, for example, in catalytic conversions. Potential applications range from catalysis to separation technology to bioengineering. Among polymeric materials, block copolymer (BCP) self-assembly is known to offer access to mesoscale-ordered structures with tunable size and morphology through control over molecular parameters such as block chemistry, sequence, and molar mass (2). Specific methods have been developed to form mesopores, including chemical block removal (3–5) and swelling with sacrificial components (6–9). The strong interest in hierarchical polymer scaffolds has resulted in specific strategies for structure generation at multiple length

scales using BCPs, such as confined self-assembly in preformed macroscale templates (10–12) and nonsolvent- or polymerization-induced phase sep-

aration (13–16). However, when combined together these approaches often require specific chemistries, only work in narrow synthesis parameter windows, or rely on multiple tedious steps that limit their general use (8). Moreover, quantitative structural assessments of nonperiodic porosity remains challenging.

A well-studied physical phenomenon in polymer science is the spinodal decomposition of polymer blends (17, 18). By driving a multi-component polymeric mixture to a supersaturated state through control of temperature or through quick solvent evaporation, a continuous interface at the micrometer scale emerges upon phase segregation. A facile and versatile, yet unexplored approach for generating hierarchical porosity would be to induce spinodal decomposition in a BCP-additive blend that would separate into an additive-rich phase and a BCP-rich phase, where one block gets selectively swollen by the additive (Fig. 1). Rinsing out both the additive-rich phase and the additive swelling of the BCP block with the same selective solvent enables hierarchical

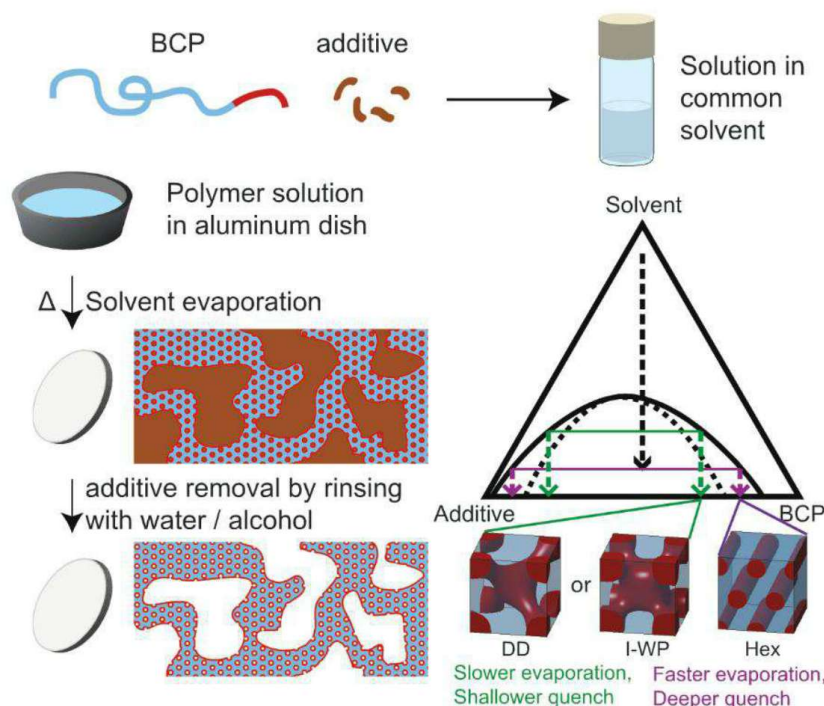


Fig. 1. Schematic for the synthesis method and ternary phase diagram. Synthesis of hierarchically porous polymer scaffolds with ordered mesostructure using the SIM²PLE method. Red color on the surface of the pores suggests PEO lining. Schematic ternary phase diagram shows paths to hexagonal and network mesostructures via solvent evaporation.

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pore formation. To render the process more relevant for applications, it is highly desirable for this solvent to be water or other protic solvents and for the swollen block to be polyethylene oxide (PEO), endowing the final material with antifouling properties well established for PEO (19, 20). Well-defined structure formation would benefit from (i) the BCP to be strongly segregating to ensure structural integrity of the BCP phase during additive removal; (ii) one block having a high glass transition temperature, T_g , to ensure mechanical stability; and (iii) a relatively small

additive to maximize BCP swelling and, in particular, its removal by rinsing.

As a first example of spinodal-decomposition-induced macro- and mesophase separation plus extraction by rinsing (SIM²PLE), we chose a widely used strongly segregating amphiphilic BCP, polystyrene-*block*-polyethylene oxide (PS-*b*-PEO), and a PEO oligomer (o-PEO), as a water- or alcohol-soluble small additive, to form a mechanically stable film through solvent evaporation-induced phase separation. A 36.6-kDa/mol PS-*b*-PEO containing 13.8 weight percent (wt %) PEO was syn-

thesized via sequential anionic polymerization according to previously reported procedures (21). The BCP was then mixed at a ratio of roughly 1:1 with the o-PEO additive with molar mass of 400 g/mol, and the mixture was dissolved in xylene at 10 total wt %, followed by solvent evaporation at 130°C on a hot plate covered with a hemispherical dome. During the evaporation period, the clear solution turned cloudy, indicating macrophase separation-induced scattering of visible light. After xylene evaporation was complete, as indicated by mass loss, the resulting white film was immersed in the protic solvents water, methanol, or ethanol to remove the o-PEO. Drying the film yielded a lightweight material with a highly opaque appearance (22).

Figure 2, A to D, shows scanning electron microscopy (SEM) images of the film cross section after removal of o-PEO via rinsing in methanol. Randomly distributed porosity is observed on the micrometer length scale throughout the film (Fig. 2, A and B). These macropores, albeit broadly distributed in size, form an interconnected network characteristic of co-continuous structures obtained via spinodal decomposition (14). Within the polymer struts, hexagonally arranged cylindrical mesopores are observed that have a radius of 10 ± 2 nm ($N = 112$), as estimated from an analysis of the SEM images (Fig. 2, C and D, and fig. S1). These mesopores are preferentially aligned parallel to the macropore walls, and a fraction of pores are observed to be accessible from the macropores (Fig. 2C). Rinsing with other protic solvents (water and ethanol) resulted in the same structures (fig. S1).

Removal of o-PEO from the bulk film is confirmed by comparing gel-permeation chromatography (GPC) results with *N,N*-dimethylformamide (DMF) as an eluent for the as-cast film and films rinsed with water, methanol, or ethanol (Fig. 2E). From the refractive index detector response, 90 to 95% of the o-PEO is removed by rinsing the as-cast film with these protic solvents for 2 hours at room temperature (compare peaks on the right at ~35 mL). Successful removal of the oligomeric additive corroborates the high degree of interconnected macro- and mesoporosity throughout the structure as observed in SEM.

Further evidence for easy accessibility of, and removal of short-chain o-PEO from, mesopores via rinsing was established via small angle x-ray scattering (SAXS). Removal of o-PEO should lead to higher electron density contrast and thus appearance of higher-order reflections. Figure 2F shows the SAXS patterns of an as-made film as well as films after rinsing with water, methanol, or ethanol. The pattern for the as-cast film shows a weak primary peak at $q^* = 0.154$ nm⁻¹; q denotes the scattering vector magnitude and is defined as $q = 4\pi\sin\theta/\lambda$, where θ is half of the scattering angle, and λ is the x-ray wavelength. After 2 hours of soaking in protic solvents at room temperature, a set of reflections consistent with a two-dimensional hexagonal lattice (*p6mm* symmetry) appears, with the identical primary

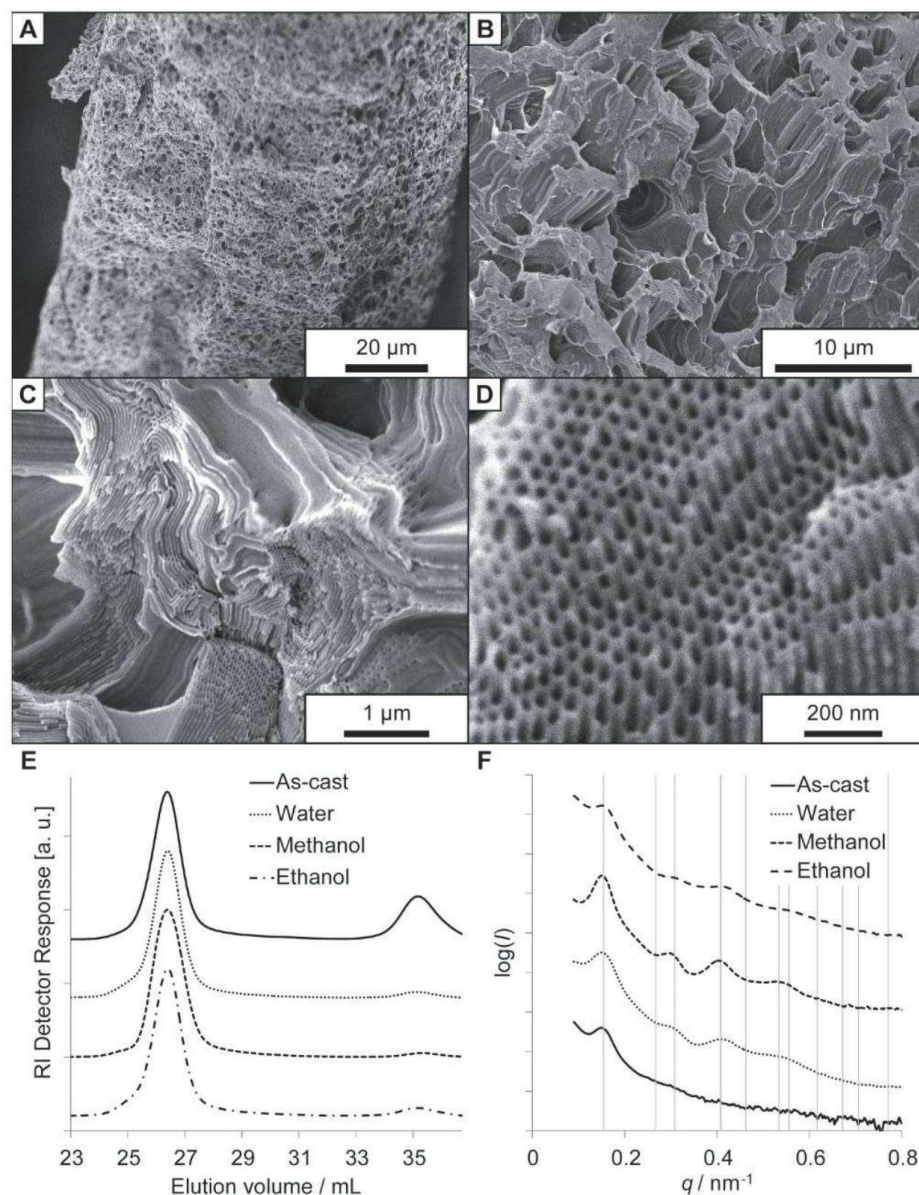


Fig. 2. Hexagonal hierarchical material characterization. (A to D) SEM images at different length scales of a fractured cross section of a bulk hierarchically porous BCP film after removal of o-PEO in methanol. (E) GPC traces of as-made and rinsed samples. Each curve is normalized in refractive index (RI) detector response at the peak height of the PS-*b*-PEO peak and calibrated for elution volume at the PS-*b*-PEO peak. a.u., arbitrary units. (F) SAXS patterns of the as-cast film and films after o-PEO removal through rinsing in protic solvents. Curves for methanol-rinsed and ethanol-rinsed samples are shifted vertically by 10^2 and 10^4 upward, respectively. Vertical lines correspond to expected peak positions for a lattice with *p6mm* symmetry with primary peak position of $q^* = 0.154$ nm⁻¹.

peak position, q^* , to the as-cast film. From the primary peak positions using $p6mm$ symmetry, a channel-to-channel distance of 47.1 nm can be calculated, suggesting substantial swelling of the PEO block by o-PEO when compared with results on the parent BCP film exhibiting a diffuse scattering peak at $q^* = 0.25 \text{ nm}^{-1}$ (fig. S2). Both GPC and SAXS results suggest that water and methanol are slightly more effective in o-PEO additive removal than ethanol, which may be due to their smaller size.

One of the advantages of working with BCPs is the versatility in precisely controlling nanostructures. We found that mesopore morphologies can be tuned by controlling the casting temperature. Figure 3, A to D, shows the macro- and mesostructural characteristics as this temperature is reduced from 130° to 100°C while using otherwise identical conditions. The low-magnification SEM image in Fig. 3A reveals the pore structure differences on the μm length scale when compared with the image in Fig. 2A. Macropores with sizes as large as 5 to 10 μm are observed for films cast at 100°C. On the mesoscale, fourfold symmetry projections for the pore arrangement in cross sections of the polymer scaffold are observed (Fig. 3, B and C), suggesting a cubic symmetry for the mesostructure formed. In contrast to the hexagonal mesopores, the cubic mesopore structures are isotropic and thus allow for even easier access from the macropores.

In order to corroborate the mesoscale structural difference observed in SEM, SAXS patterns were obtained for the films cast at 100°C (Fig. 3D). Similar to the films prepared at 130°C, SAXS patterns of as-cast samples showed weak peaks, whereas after rinsing with protic solvents patterns exhibited strong higher-order scattering peaks while retaining the primary peak position of the as-cast film. For example, after rinsing with methanol, films show a set of reflections with the ratios of $(q/q^*)^2 = 1, 3/2, 4/2, 6/2, 9/2, 10/2, 12/2, 14/2$, and $17/2$ and the first-order peak location at $q^* = 0.183 \text{ nm}^{-1}$, which is consistent with a cubic symmetry of aspect 4 with lattice spacing of 48.7 nm (23), typically associated with a double diamond (DD) network morphology. We used transmission electron tomography to generate a 3D reconstruction of the mesostructure (Fig. 3, E and F, and fig. S3). The reconstruction shows a cubic periodic network structure most consistent with either DD or Schoen's I-WP morphology (Fig. 1) but did not enable a definite assignment (22). Although the packing frustration in fourfold (DD) or eightfold (I-WP) nodes generally precludes such highly crowded network structure formation in neat BCP systems, BCP/homopolymer blends are predicted by self-consistent field theory to form DD structures through homopolymer segregation in the nodes (24–26).

We have applied the SIM²PLE method to a variety of systems. We first varied the solvent from xylene to anisole, a more-polar and hydrogen-bonding solvent that dissolves PEO better and has a higher boiling point. The resulting meso-

structure (fig. S4) is similar to that obtained from xylene, indicating that the mesoporous structures for this pair of solvents are not as sensitive to the solvent choice as they are to the change in casting temperature. We have further varied the BCP system from PS-*b*-PEO to poly(4-*tert*-butyl)styrene-*block*-polyethylene oxide (PtBS-*b*-PEO) and to the triblock terpolymer polyisoprene-*block*-polystyrene-*block*-polyethylene oxide (PI-*b*-PS-*b*-PEO). For PtBS-*b*-PEO, preliminary results of the SIM²PLE method with oligomeric poly(acrylic acid) as the additive and tetrahydrofuran as solvent gave similar hierarchical structures and accessible pores after rinsing with ammonium hydroxide-containing aqueous solution (fig. S5).

The higher glass-transition temperature of PtBS ($T_g = 138^\circ\text{C}$) relative to PS ($T_g = 100^\circ\text{C}$) allows for standard sterilization procedures, for example, autoclaving, to be used on these materials for potential biological applications. Successful translation to PI-*b*-PS-*b*-PEO (fig. S6) suggests a path to membranes with improved toughness (15). Last, preliminary experiments with a 226-kg/mol molar mass PS-*b*-PEO-L yielded highly pliable films with multiscale porosity, suggesting that this method can also be translated to highly entangled polymers (fig. S7).

Nanoscale x-ray computed tomography, nanoCT, was used to image the nonperiodic 3D macroporous structure on micrometer length scales.

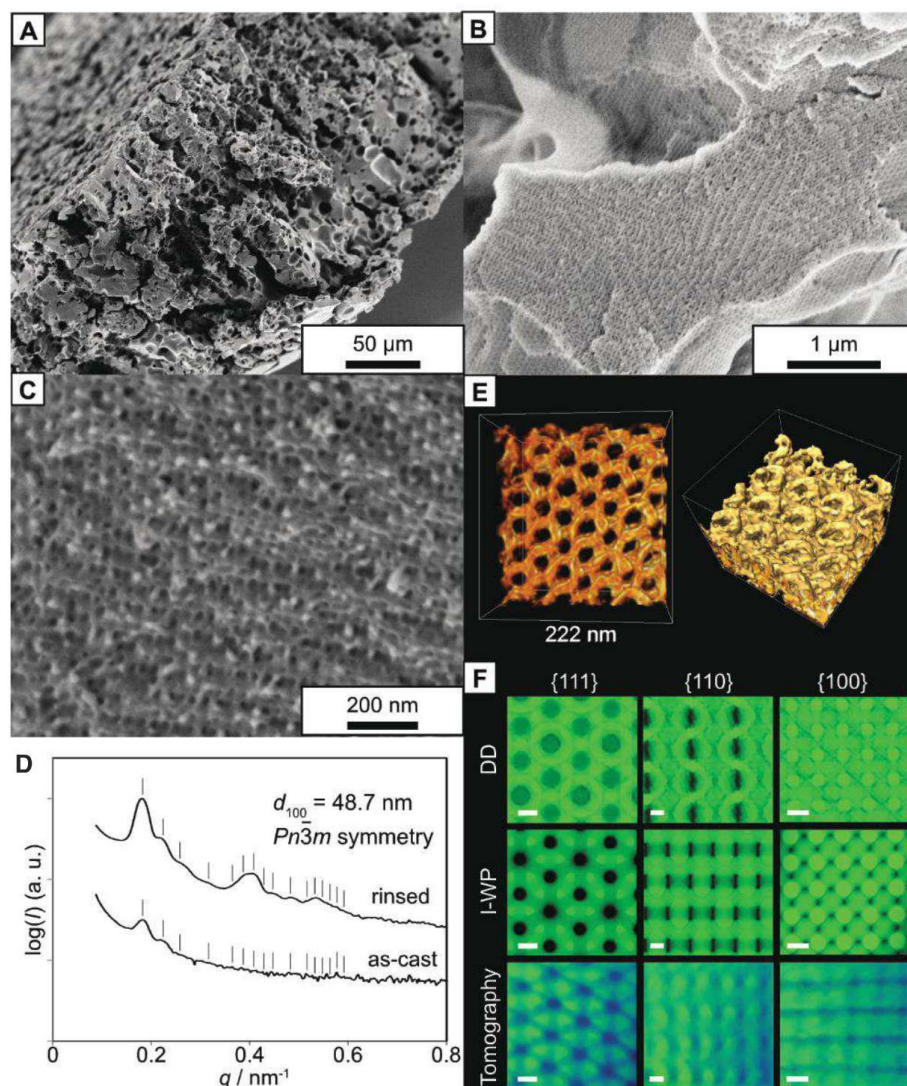


Fig. 3. Cubic hierarchical material characterization. (A to C) SEM images of film cross sections of PS-*b*-PEO/o-PEO blends cast from xylene at 100°C at increasing magnifications. Images show macroporosity (A), interconnected mesopores accessible from the macropores (B), and fourfold symmetry in the mesoscale porosity (C). (D) SAXS patterns of as-made and rinsed film cast at 100°C. Spectrum for the rinsed film is shifted in intensity compared with results from as-made films. Tick marks correspond to expected peak positions for a lattice with $Pn\bar{3}m$ symmetry with the first peak position corresponding to (110) reflection at 0.183 nm^{-1} . (E) TEM tomographic reconstruction of the polymer scaffold. The material is shown in bright colors. (F) Representative projection images generated from proposed DD and I-WP structure models and tomographic reconstruction. Scale bars indicate 20 nm.

This technique requires no alteration to the samples before imaging and can reconstruct a relatively large volume of $\sim 1 \text{ mm}^3$. Figure 4A shows a 3D rendering of x-ray absorption contrast in a PS-*b*-PEO/o-PEO-based sample cast from xylene at 130°C with hexagonal mesostructure, revealing the co-continuous nature of the macrostructure. Strut-thinning processes (27) on the interface toward either the polymer region or toward the pore region yielded fully connected skeletal networks throughout the film thickness, confirming a micrometer-scale bicontinuous net-

work consistent with the suggested spinodal decomposition mechanism (Fig. 4B). A characteristic feature size of 5 to 10 μm is detected from the node-to-node distance distribution of the skeletal network as well as from a weak correlation peak at 7.6 μm in the radial distribution plot of the 3D fast Fourier transform (FFT) (Fig. 4, C and D). Furthermore, the skeletal network analysis provides information on the degree of complexity in the population distribution of struts per node shown in Fig. 4E: Although a large fraction of nodes are found to be trivalent, we also observed

nodes that are more crowded. Similar analysis was performed on a sample cast at 100°C with cubic mesostructure (fig. S8). The distribution of struts per node shifts to a larger number for the 100°C sample compared with the 130°C sample. Accessibility of, and transport through, network pores is of critical importance to structure replication processes (28). Figure 4, F and G, shows SEM images of calcium carbonate (calcite) crystallized within the hierarchical scaffold after template removal (22). Whereas the image in Fig. 4F depicts typical facets of calcite, the Fig. 4G image shows mineral replica features consistent with growth in macro- and hexagonally arranged cylindrical mesopores. Growth into the template on both length scales has occurred over hundreds of nanometers, implying transport of crystal precursors through the film.

The presence of o-PEO played a dual role in the formation of film porosity. We speculate that the o-PEO, a precipitating solvent for the majority PS block of the BCP, induces spinodal decomposition of the initially single-phase solution as the solvent evaporates, consistent with the observed opacity in the films. Preliminary mixing experiments of PS-*b*-PEO and o-PEO in xylene suggest that the miscibility gap extends far out on the side of the additive and goes up to about 40 wt % solvent (Fig. 1) (22). The macrophase separated o-PEO provides continuous macroporous domains. Evaporation at different temperatures leads to a different quench depth into the spinodal decomposition region, resulting in different amounts of residual o-PEO in the PEO block. Deeper quenches (higher T , faster evaporation) lead to less additive in the BCP-rich domains (Fig. 1), resulting in a change in ordered mesostructure, for example, from network to hexagonal, consistent with our experimental observations (29, 30). Because o-PEO is not a strongly associating swelling agent, rinsing at room temperature for a short period of time is sufficient to form the final hierarchically porous structure. This is in contrast to the often harsh bond-cleaving conditions required for etching block copolymer domains (3–5). Use of highly amphiphilic BCPs such as PS-*b*-PEO, PtBS-*b*-PEO, or PI-*b*-PS-*b*-PEO prevents substantial intrusion of rinsing solvents into the hydrophobic part of the scaffold, contributing to mesostructural integrity. We also note that, because the PEO block in the BCP is not decomposed, the pores are lined with PEO chains, providing wettability and possibly anti-fouling properties of the walls.

We have developed a facile and versatile one-pot approach for the preparation of hierarchical macro- and mesoporous polymer scaffolds via spinodal decomposition of BCP/small additive mixtures from solution and have introduced techniques for their quantitative structural characterization. The SIM²PLE method combines ease of preparation with high degree and choice of ordering within the macroporous structure. It further replaces more demanding decomposition or chemical transformation steps to induce macro- and

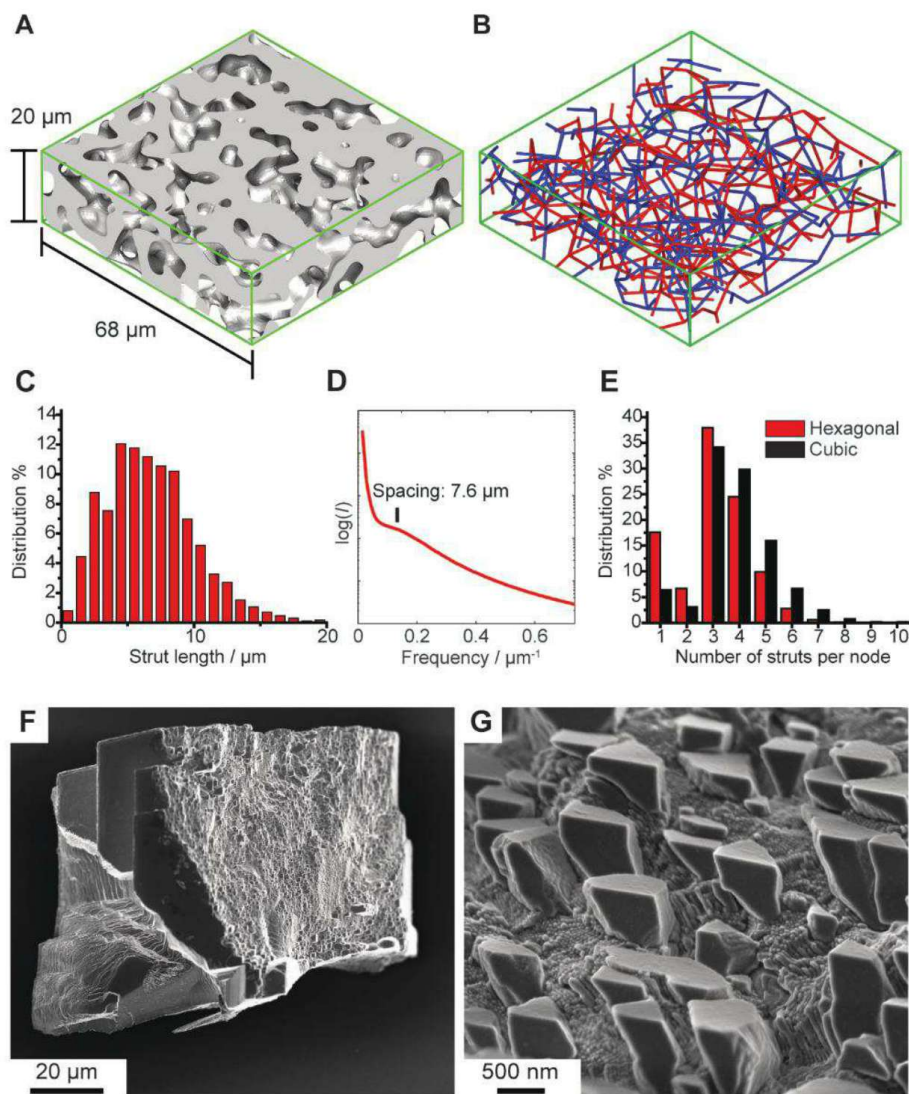


Fig. 4. Macrostructure characterization and materials use as scaffold. (A to E) A 3D tomographic reconstruction of the macrostructure of a film of system PS-*b*-PEO/o-PEO/xylene/130°C with hexagonal mesostructure, using nanoCT. (A) Isosurface visualization. (B) Skeletal networks for (A) of the polymeric (blue) and the porous (red) regions. (C) Node-to-node distance distribution of the porous network from a 136- μm cubic region. (D) Radial distribution function of the 3D FFT volumetric data from (C). (E) Population distribution of struts per node for (C) (red columns), as well as an identically generated network of a sample cast at 100°C with cubic mesostructure (black columns). Mono- and divalent nodes arise from analysis artifacts on the edges of the volume. (F) Low-magnification SEM image showing the rhombohedral facets consistent with the crystal habit of the calcite polymorph. Right-hand portion of the crystal with the highly textured surface was grown in contact with the polymer scaffold. (G) High-magnification image of the mineral replica of the polymer. The larger features, associated with growth into macropores, show {104} facets, which are all uniformly aligned, indicating an epitaxial relationship to the parent crystals.

mesoporosity by a simple rinsing step with protic solvents like water or alcohols. It thus provides advantages over multiple-step fabrication methods currently used for integrating nanoscale porosity into macroscopic scaffolds. We have already shown it to work for different BCPs, small molar mass additives, solvents, and protic rinsing agents. Because the method is based on general thermodynamic principles, it may provide a powerful conceptual approach to generate hierarchical materials.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S9

Table S1

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Movies S1 and S2

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Liquid-Mediated Dense Integration of Graphene Materials for Compact Capacitive Energy Storage

Xiaowei Yang, Chi Cheng, Yufei Wang, Ling Qiu, Dan Li*

Porous yet densely packed carbon electrodes with high ion-accessible surface area and low ion transport resistance are crucial to the realization of high-density electrochemical capacitive energy storage but have proved to be very challenging to produce. Taking advantage of chemically converted graphene's intrinsic microcorrugated two-dimensional configuration and self-assembly behavior, we show that such materials can be readily formed by capillary compression of adaptive graphene gel films in the presence of a nonvolatile liquid electrolyte. This simple soft approach enables subnanometer scale integration of graphene sheets with electrolytes to form highly compact carbon electrodes with a continuous ion transport network. Electrochemical capacitors based on the resulting films can obtain volumetric energy densities approaching 60 watt-hours per liter.

Electrochemical capacitors (ECs) store energy by charging electrical double layers through highly reversible ion adsorption on the surface of high-surface-area electrodes, generally made from porous carbon (1–3). They are attractive for energy storage because of their fast charging capability and long life span (4, 5). The energy density (or the amount of energy stored per unit volume) of most commercially available ECs to date is close to 5 to 8 Wh/L (6), still

much lower than that of lead-acid batteries (50 to 90 Wh/L) (7).

The efficiency of a material for EC energy storage can be described by its specific volumetric capacitance in a single electrode (C_{vol}) and energy density against the volume of two EC electrodes ($E_{\text{vol-electrode}}$); the volumetric energy density against the whole EC stack ($E_{\text{vol-stack}}$)—including two electrodes, electrolyte, a separator between two electrodes, and current collectors—was recently recommended to be a more reliable parameter than the gravimetric one to evaluate the real potential of a porous carbon for ECs (8–10). $E_{\text{vol-stack}}$ relates to gravimetric capacitance of the active carbon component in a single electrode

($C_{\text{wt-C}}$), packing density of the carbon (ρ), volume fraction of the electrodes in the device stack ($f_{\text{electrode}}$), as well as the nominal voltage (U) of the EC as follows (6).

$$C_{\text{vol}} = C_{\text{wt-C}} \times \rho \quad (1)$$

$$E_{\text{vol-electrode}} = \frac{C_{\text{vol}} \times U^2}{8} \quad (2)$$

$$E_{\text{vol-stack}} = E_{\text{vol-electrode}} \times f_{\text{electrode}} \quad (3)$$

Improvement in $E_{\text{vol-stack}}$ requires that $C_{\text{wt-C}}$, ρ , and $f_{\text{electrode}}$ of electrodes all be maximized, but increasing ρ and $f_{\text{electrode}}$ (or the thickness of electrodes) is difficult without substantially compromising the $C_{\text{wt-C}}$. As an example, graphite is possibly the most compact conductive carbon materials (~2.2 g/cm³ at the ambient condition) but delivers little capacitance because ions cannot access the interplanar space. Even if the packing density of the graphene stack was reduced by half to 1.1 g/cm³, the interplanar spacing would be only ~0.67 nm (fig. S1). The ion diffusion and adsorption in such small-size channels is very sensitive to pore size, surface wettability, and particularly the pore interconnectivity (4, 11, 12). Impaired ion transport in subnanometer pores becomes even more pronounced when the electrodes are made thicker, further limiting the achievable value of $E_{\text{vol-stack}}$ (13, 14). Although progress has been made to achieve high $C_{\text{wt-C}}$ values through a variety of methods, such as laser scribing or chemical activation of graphene (15–19), the packing density

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of the electrodes obtained was rather low, ranging from 0.05 to 0.75 g/cm³ (8–10).

Recent advances in graphene chemistry (20, 21), particularly the colloidal chemistry of chemically reduced graphene oxide (22), also called chemically converted graphene (CCG), open up new ways to address this challenge. We have shown that CCG can be well dispersed in water without the need for any surfactants by controlling its colloidal chemistry (23). The resulting CCG sheets, being microscopically corrugated (22), could self-assemble to form an oriented hydrogel film through a simple directional-flow-induced bottom-up assembly process (24). As a result of micro-corrugation and repulsive intersheet solvation/electrostatic forces, CCG sheets in the hydrogel film remained largely separated, which gave rise to a high C_{wt-C} of over 200 F/g (25). However, the as-formed gel film exhibited a low packing density (~ 0.069 g/cm³), resulting in a mediocre C_{vol} (~ 18 F/cm³).

In this work, we show that in contrast to porous carbon films prepared by the traditional techniques that generate fixed pore size and “hard” texture (26), the CCG hydrogel films, with a metastable and adaptive pore structure, can be compressed irreversibly by capillary pressure to increase the packing density through controlled removal of volatile solvent trapped in the gel. The

graphene sheets in the films stacked in a nearly face-to-face fashion, so the packing density can be increased up to ~ 1.33 g/cm³, nearly double that of the traditional activated porous carbon (0.5 to 0.7 g/cm³) (6). More important, the liquid electrolyte-mediated CCG (EM-CCG) films created a continuous ion transport network that led to exceptionally high C_{vol} and $E_{vol-stack}$.

The CCG hydrogel films obtained by filtration of CCG dispersion were exchanged with a miscible mixture of volatile and nonvolatile liquids and were then subjected to removal of the volatile liquid by vacuum evaporation (fig. S2) (27). The gel film reduced in the thickness direction as a result of selective removal of the volatile liquid, while the CCG sheets remained solvated by the nonvolatile liquid during the whole process. The packing density of these flexible films (Fig. 1A) was controlled from 0.13 to 1.33 g/cm³ by changing the ratio of volatile and nonvolatile liquids (Fig. 1D and fig. S9G). The nonvolatile liquid electrolytes, sulfuric acid, and 1-ethyl-3-methylimidazolium tetrafluoroborate (EMIMBF₄) were used in these studies.

Scanning electron microscopy (SEM) analysis revealed that the as-prepared EM-CCG films had a rather uniform cross section (Fig. 1, B and C, and figs. S3 and S9), which was also confirmed by energy dispersive x-ray spectroscopy

(EDX) mapping of sulfur (fig. S3). The thicknesses were nearly proportional to the volumetric fraction of incorporated nonvolatile liquids trapped in the gels. X-ray diffraction (XRD) analysis showed that the as-compressed EM-CCG films displayed a nearly amorphous structure (fig. S4). Only a small and broad peak at around 23°, corresponding to a d_{002} distance of 0.39 nm, was detected when $\rho > 0.76$ g/cm³ but the peak was much weaker than that of the dried CCG film. This indicated that the majority of CCG sheets did not restack back to graphite, despite being substantially compressed.

To investigate the effect of ρ on capacitive energy storage, we fabricated a series of prototype ECs with the same areal mass loading of CCG sheets (27). Fully dried CCG films with a ρ of 1.49 g/cm³ were also tested for comparison. Figure 2 presents typical EC characterization of EM-CCG film-based ECs in 1.0 M H₂SO₄ electrolyte. The cyclic voltammetry (CV) curves (Fig. 2, A and B) showed nearly symmetrical rectangular shapes, indicative of an ideal capacitive behavior. As shown in Fig. 2C, a higher ρ led to a lower C_{wt-C} at a given charging rate. However, the extent of decrease in capacitance was strongly dependent on whether the CCG film was preincorporated with an electrolyte during the preparation process of the film. At a low operation rate of 0.1 A/g, when ρ of the EM-CCG films was increased from 0.13 to 1.33 g/cm³, C_{wt-C} only dropped from 203.2 to 191.7 F/g (fig. S6A). In contrast, the completely dried CCG film (1.49 g/cm³) only gave a C_{wt-C} of 155.2 F/g. The difference became even more pronounced when the films were more rapidly charged (fig. S6, B to D). All of the EM-CCG films delivered a $C_{wt-C} > 100$ F/g at an operation rate of 100 A/g, whereas the dried CCG film yielded only 10.2 F/g. The data obtained with an organic electrolyte [EMIMBF₄/acetonitrile (AN)] at an operation voltage of 3.5 V showed a similar result (figs. S10 and S11).

The influence of ρ on C_{vol} was very different. The C_{vol} of the EM-CCG films was nearly proportional to ρ (Fig. 2D and figs. S11E and S13). The highly compact EM-CCG films (1.25 to 1.33 g/cm³) yielded a C_{vol} of 255.5 F/cm³ in aqueous electrolyte and 261.3 F/cm³ in organic electrolyte at 0.1 A/g. These values were much higher than those of the existing porous carbon materials (table S2). The EM-CCG films showed a very high C_{vol} at a broad range of charging rates from 0.1 (0.133 A/cm²) to 200.0 A/g (266.0 A/cm²). Even though the dried CCG film was able to deliver a C_{vol} of around 231.5 F/cm³ at 0.1 A/g, the value decreased rapidly with the increase of operation rate (Fig. 2D) as a result of a rapidly declining C_{wt-C} .

The above results showed that preincorporation of electrolytes in the CCG films ensured excellent rate performance of compact EM-CCG films. We performed EC impedance spectroscopy analysis to further ascertain the role of the preincorporated electrolyte. A frequency response analysis at open circuit potential over the frequency

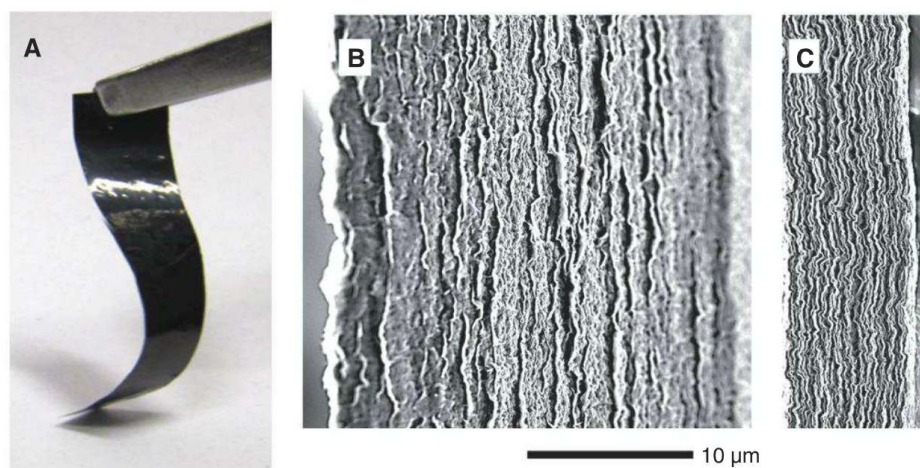
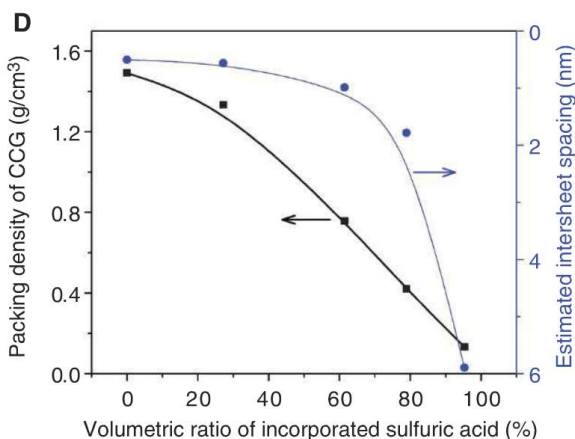


Fig. 1. Characterization of liquid electrolyte-mediated CCG (EM-CCG) films. (A) A photograph showing the flexibility of the film. (B and C) SEM images of cross sections of the obtained EM-CCG films containing (B) 78.9 volume percent (vol. %) and (C) 27.2 vol. % of H₂SO₄, respectively, corresponding to $\rho = 0.42$ g/cm³ and $\rho = 1.33$ g/cm³. (D) The relation between the volumetric ratio of incorporated electrolyte and the packing density as well as the estimated intersheet spacing.



range from 100 kHz to 10 mHz yielded the Nyquist plots shown in Fig. 2E. The plot featured a vertical curve, indicating a nearly ideal capacitive behavior of the cell. At high-frequency regions, a transition from a vertical curve feature to a -45° line followed by a semicircle was observed, and this transition was pushed to higher-frequency regions as a decrease in the packing density of EM-CCG film. This variation was small between the different EM-CCG films, but it showed a pronounced difference when they were compared with the dried one. The corresponding time constant τ_0 (the inverse of the characteristic frequency at which -45° is reached in the Bode phase plots) also responded in a similar fashion (Fig. 2F). The τ_0 increased from 0.51 to 0.73 s as the ρ of the EM-CCG films increased from 0.76 to 1.33 g/cm³. A doubling of ρ only led to a change in τ_0 by a factor less than

1.45. By contrast, less than 20% change in ρ from the EM-CCG film (1.33 g/cm³) to the dried film (1.49 g/cm³) gave a shift of τ_0 by a factor more than 5.27. The frequency response of the CCG films in organic electrolyte followed a similar trend (fig. S10C).

The above results indicated that highly efficient ion transport channels were retained in the EM-CCG films, even when they were packed quite densely. Due to the fluid nature of liquid electrolytes, the continuous liquid network was likely to remain within the whole film during the capillary compression process. Furthermore, as the electrolyte became integrated within the film from the start of the assembly process, there was no subsequent wettability issue for these EM-CCG films, which remained a serious problem for the dried CCG film (28).

The indispensable role of the preincorporated electrolyte in ion transport was further evidenced by the effect of areal mass loadings (or electrode thickness) on the electrochemical performance (Fig. 3, A and B, and fig. S7). Both C_{wt-C} and C_{vol} generally decreased with increasing thickness of the electrodes (4, 13, 29); this effect was particularly prominent when the pore size was in the subnanometer range (13). Figure 3 showed how the EC performance of the EM-CCG film ($\rho = 1.25$ g/cm³) and the dried CCG film ($\rho = 1.49$ g/cm³) responded to mass loading in the organic electrolyte. As with other porous carbon (13), both the C_{vol} and $E_{vol-electrode}$ of the two types of materials decreased with thickness. Nevertheless, the EM-CCG film displayed a much slower rate of decrease than the dried film, reflecting superior ion transport. When the effect

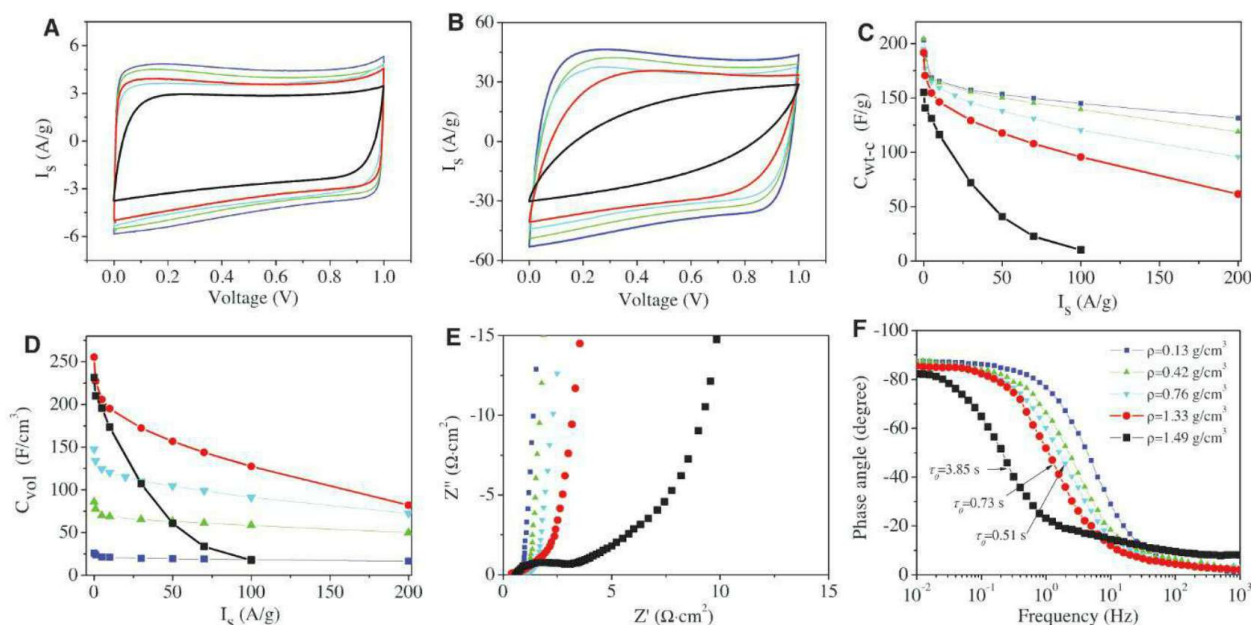


Fig. 2. Electrochemical characterization of EM-CCG films. Electrochemical characterization of EM-CCG films in 1.0 M H₂SO₄ ($\rho = 0.13, 0.42, 0.76$, and 1.33 g/cm³, respectively). CV curves at (A) 50 mV/s and (B) 500 mV/s. (C) Gravi-

metric and (D) volumetric capacitances with varied charging/discharging current densities. (E) Nyquist plots and (F) Bode plots of phase angle versus frequency. The results for the dried CCG film ($\rho = 1.49$ g/cm³) are also shown for comparison.

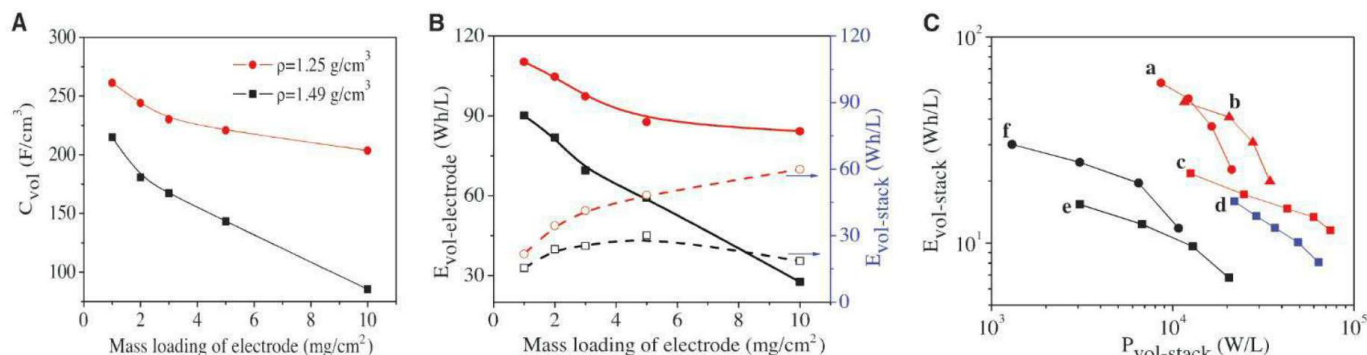


Fig. 3. Volumetric performance of ECs based on EM-CCG film electrodes. (A) Volumetric capacitance and (B) energy density as a function of the areal mass loading of EM-CCG film ($\rho = 1.25$ g/cm³) and the dried CCG film ($\rho = 1.49$ g/cm³) at the current density of 0.1 A/g. (C) Ragone plots of representative EM-CCG films showing how $E_{vol-stack}$ and $P_{vol-stack}$ depend on both the packing density and areal

mass loading of CCG: (a) 1.25 g/cm³, 10.0 mg/cm²; (b) 1.25 g/cm³, 5.0 mg/cm²; (c) 1.25 g/cm³, 1.0 mg/cm²; (d) 0.39 g/cm³, 1.0 mg/cm²; the dried CCG films (1.49 g/cm³) were also presented for comparison with the areal mass loading of (e) 1.0 mg/cm² and (f) 5.0 mg/cm². The data were obtained from the prototype ECs with EMIMBF₄/AN as electrolyte and an operation voltage of 3.5 V.

of thickness on $E_{\text{vol-stack}}$ was considered, the volume fraction of the active electrodes ($f_{\text{electrode}}$) needed to be taken into account (see Eq. 3). Because the $f_{\text{electrode}}$ increased with the electrode thickness and the other components of ECs remained unchanged, the reduction in $E_{\text{vol-electrode}}$ with thickness was not substantial in the case of the EM-CCG film; however, the EM-CCG film displayed an overall increase in $E_{\text{vol-stack}}$ with thickness (see the red dashed line in Fig. 3B). The EC device based on two CCG/electrolyte films of 80 μm (containing 10 mg/cm^2 of CCG, comparable to the amount of carbon contained in many commercial EC devices) yielded an $E_{\text{vol-stack}}$ of 59.9 Wh/L. By contrast, the $E_{\text{vol-stack}}$ of the dried CCG film initially increased with thickness, then decreased by a mass loading of 5 mg/cm^2 because of substantial drop in $E_{\text{vol-electrode}}$ (see the black dashed line in Fig. 3B). The maximum $E_{\text{vol-stack}}$ using 10 mg/cm^2 of the dried CCG film as electrodes was only ~ 18.4 Wh/L, further highlighting the crucial role of low ion transport resistance for achieving a high $E_{\text{vol-stack}}$.

The ECs based on our compact EM-CCG films also delivered a high volumetric power density. As shown in the Ragone plots (Fig. 3C), both the $E_{\text{vol-stack}}$ and the $P_{\text{vol-stack}}$ (maximum power density) were strongly dependent on the packing density and areal mass loading of CCG. The $P_{\text{vol-stack}}$ corresponding to the highest $E_{\text{vol-stack}}$ (59.9 Wh/L) was ~ 8.6 kW/L. With the same areal mass loading of 1 mg/cm^2 , EM-CCG film (1.25 g/cm^3) could deliver the maximum power density of ~ 75 kW/L, higher than that of the dried CCG film (1.49 g/cm^3) as well as the low packing-density EM-CCG film (0.39 g/cm^3). These results also suggested that the key technical specifications

of CCG-based ECs (e.g., $E_{\text{vol-stack}}$, $P_{\text{vol-stack}}$, and τ_0) could be readily customized to suit different applications by simply tuning the packing density of the CCG electrodes.

These EM-CCG films were very stable under repeated charging/discharging or under the application of a constant voltage (figs. S8 and S12). Over 95% of the initial capacitance was retained after a 300-hour constant voltage holding at 3.5 V in a neat EMIMBF₄ electrolyte (fig. S12). In addition, the fabrication of CCG gel films and subsequent compression are essentially compatible with the traditional cost-effective paper-making process and can be readily scaled up. All these attractive features make this class of graphene materials promising for large-scale real-world applications.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S13
Tables S1 and S2
References (30–39)

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Detection of a Spinning Object Using Light's Orbital Angular Momentum

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The linear Doppler shift is widely used to infer the velocity of approaching objects, but this shift does not detect rotation. By analyzing the orbital angular momentum of the light scattered from a spinning object, we observed a frequency shift proportional to product of the rotation frequency of the object and the orbital angular momentum of the light. This rotational frequency shift was still present when the angular momentum vector was parallel to the observation direction. The multiplicative enhancement of the frequency shift may have applications for the remote detection of rotating bodies in both terrestrial and astronomical settings.

The spin angular momentum of light is manifested as circular polarization and corresponds to the spin angular momentum of

the photon, $\hbar$ (Planck's constant divided by 2π). More than 20 years ago, it was recognized that light beams with a helical phase structure described by $\exp(i\ell\phi)$, where ℓ is an integer and ϕ is the azimuthal coordinate, also carry an orbital angular momentum corresponding to $\ell\hbar$ per photon (*1*). Since then, this orbital angular momentum (OAM) has been studied in various contexts such as optical micro-manipulation and quantum optics (*2*).

Consideration has been given to the use of OAM in imaging and remote sensing, where the detection of the angular momentum may reveal the structure or potentially the motion of the object (*3–7*). When light is scattered from a spinning object, we find that the rotation rate of the object can be measured by analyzing frequency shifts in the OAM of the light. This method of remote sensing has applications in both terrestrial and astronomical arenas.

The Doppler shift is a well-known phenomenon in which the relative velocity v between a wave-emitting source and an observer gives a frequency shift Δf of that wave. Such an effect is readily seen for audio waves, where the pitch of the sound changes with the speed of the source. For a light beam, the resulting frequency shift is $\Delta f = f_0 v/c$, where f_0 is the unshifted frequency and c is the speed of light. Less well-known than this linear effect is the rotational, or angular, Doppler effect (*8–11*). This frequency shift has also been considered for the scattering of light from atomic (*12*) or macroscopic (*13*) objects rotating around the axis of a helically phased laser beam. For a beam with helical phase fronts, a rotation of angular frequency

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Ω between the source and observer shifts the frequency by

$$\Delta f = \frac{(\ell + \sigma)\Omega}{2\pi} \quad (1)$$

where $\sigma = \pm 1$ for right- and left-handed circularly polarized light and 0 for linearly polarized light, hence $(\ell + \sigma)\hbar$ is the total angular momentum per photon (14). All of this previous rotational work has been based on pure OAM states, explicitly rotated using specialist optical elements.

The standard linear Doppler shift applies when the relative motion between source and observer is along the direction of observation. For motion transverse to the direction of observation, a reduced Doppler shift can still be observed in the light scattered at an angle α from the surface normal. For small values of α , this reduced Doppler shift is given by

$$\Delta f = \alpha \frac{f_0 v}{c} \quad (2)$$

This frequency shift occurs because the form of the scattered light is not determined solely by the laws of reflection. The roughness of the surface means that some of the light normally incident on the surface is scattered at angle α . The observation of this frequency shift in light scattered from a moving object is the basis of speckle (i.e., laser Doppler) velocimetry, and it is used for the remote sensing of the transverse velocity of moving surfaces (15) or fluids (16) (Fig. 1A).

It is also possible to understand speckle velocimetry in the time domain. In illumination mode, the resulting interference between the two beams at $\pm\alpha$ creates straight-line fringes with period $\Lambda = \lambda/2\alpha$ (small α , where $\sin \alpha \approx \alpha$). When a rough surface translates across this fringe pattern, the inhomogeneity of the surface results in a slight modulation in the intensity of the scattered light. The frequency of this modulation is $f_{\text{mod}} = v/\Lambda$, which is exactly the same rate as anticipated from the differential Doppler shifts of the two beams given by Eq. 2. The depth of the modulation depends on the period of the fringes relative to the period of the surface roughness, where the depth is maximized when the two periods match. These two complementary explanations of speckle velocimetry have an angular equivalent.

In a helically phased beam, the Poynting vector, and hence the optical momentum, has an azimuthal component at every position within the beam. The angle between the Poynting vector and the beam axis is $\alpha = \ell \lambda/2\pi r$, where r is the radius from the beam axis (17) (Fig. 1B). In the frequency domain, for a helically phased beam illuminating a spinning object, we can see from Eq. 2 that the Doppler frequency shift of the on-axis ($\ell = 0$) scattered light is given as

$$\Delta f = \frac{\ell\Omega}{2\pi r} \left(f_0 \frac{\Omega r}{c} \right) = \frac{\ell\Omega}{2\pi} \quad (3)$$

Note also in Eq. 3 that there is no dependence on polarization because, unlike the OAM, the

polarization is largely unchanged by the process of scattering.

When the illumination comprises two helically phased beams of opposite values of ℓ , their scattering into a common detection mode gives opposite frequency shifts and an intensity modulation of frequency

$$f_{\text{mod}} = \frac{2|\ell|\Omega}{2\pi} \quad (4)$$

If the common detection mode is not $\ell = 0$, then the frequency shifts of the two beams are different, but

their frequency difference remains the same. Hence, even if the detection is multimodal, all of the detected modes experience the same modulation frequency.

Within the time domain, the interpretation is that the superposition of two helically phased beams with opposite values of ℓ creates a beam cross section with a modulated intensity of 2ℓ radial petals (18). Therefore, the light scattered from the rough surface of a spinning disc will undergo an intensity modulation also given by Eq. 4.

Linear optical systems tend to be reciprocal in that the source and detector can be interchanged.

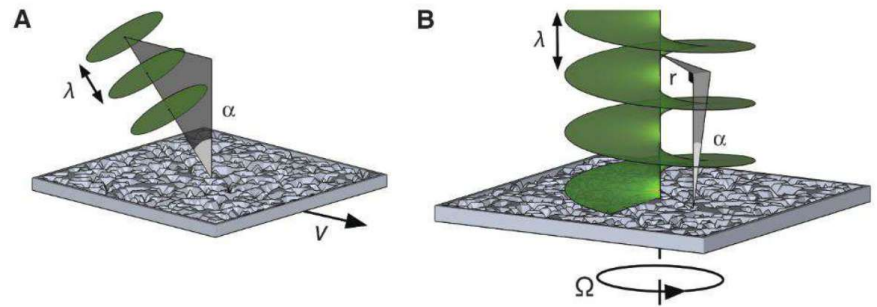


Fig. 1. Doppler shift. Light scattered from a moving surface can be Doppler-shifted in frequency. This frequency shift can be observed for (A) translation and (B) rotation.

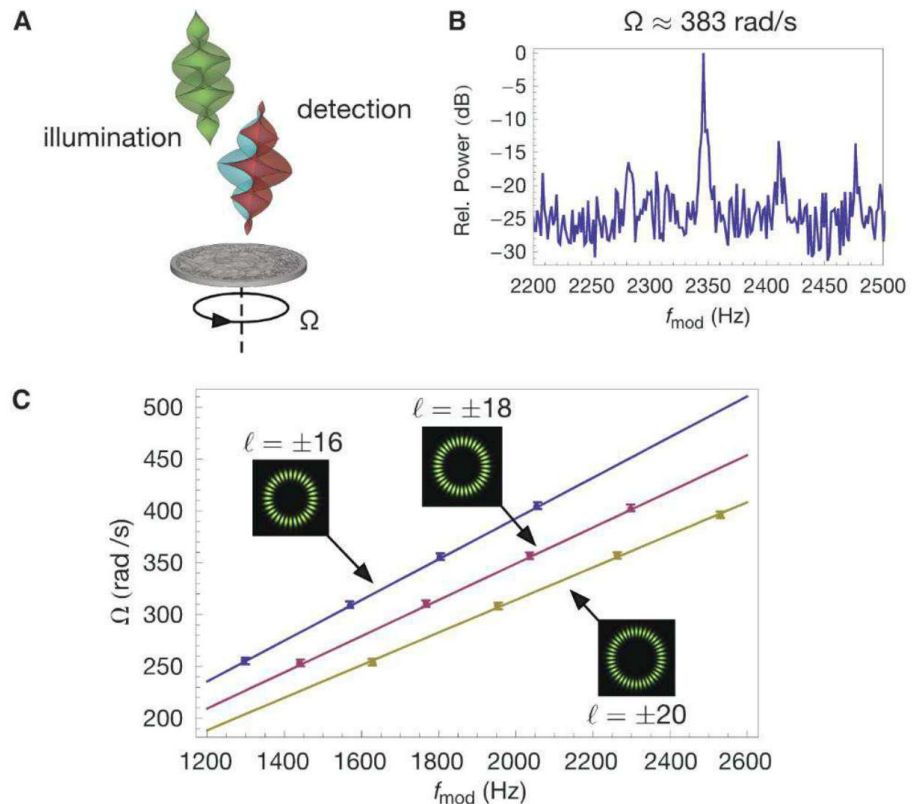


Fig. 2. Rotational Doppler shift. (A) A superposition of helically phased beams with opposite signs of ℓ , incident on a surface rotating at a speed Ω , results in a Doppler shift of the on-axis scattered light. The size of this shift is dependent on the value and sign of ℓ . For a given input superposition, shown in green, the light scattered from the positive ℓ beam will be blue-shifted and that from the negative ℓ beam will be red-shifted. (B) This differential shift will result in an intensity modulation at a particular frequency, $f_{\text{mod}} = 2346 \pm 1$ Hz. (C) Values of f_{mod} were measured for different rotation speeds and values of $|\ell|$, shown as points, and were compared to the values predicted from Eq. 4, shown as solid lines.

Consequently, we would expect the frequency shift produced by the spinning surface to be observed either in the case of illumination by a beam containing OAM and the on-axis detection of the scattered light, or for on-axis illumination and detection of an OAM component in the scattered light.

For illumination of the spinning object with light of specific OAM modes, a diode laser at 670 nm is coupled to a single-mode fiber, the output of which is collimated and used to illuminate a phase-only spatial light modulator (SLM). The SLM is programmed with a kinoform to produce a superposition of two helically phased beams with opposite signs of ℓ . The phase contrast is adjusted over the SLM cross section such that the radial intensity structure of the diffracted beam is a single annulus corresponding to a $p = 0$ Laguerre-Gaussian mode (19). The plane of the SLM is reimaged using an afocal telescope to illuminate the spinning object. Relay mirrors allow the axis of the illuminating beam to be precisely aligned to the rotation axis of the object. The diameter of the beam superposition on the object is about 18 mm, which gives a petal period of about 2 mm for typical values of $\ell = \pm 18$. This petal beam illuminates a metallic surface attached to a plastic rotor, which is driven at speeds ranging from 200 to 500 radians/s. The rough nature of the surface means that irrespective of the modal composition of the illumination light, the light scattered from the surface comprises a very wide range of modes. The modal bandwidth of this scattered light is a function of both the size of the

illuminating beam and the range of angles over which the light is scattered or, more important, detected. The bandwidth is usefully approximated in terms of the Fresnel number of the optical system. A lens and a large-area photodiode are used to collect light scattered from the spinning surface. The output of the detector is digitized and Fourier-transformed to give the frequency components of the detected intensity modulation (see supplementary materials).

For the light scattered from the rotating surface when illuminated with a Laguerre-Gaussian superposition of $\ell = \pm 18$ (Fig. 2B), the resulting power spectra were obtained from a data collection period of 1 s. A clearly distinguishable peak was observed at a frequency matching that predicted by Eq. 4. To further test the relationship predicted in Eq. 4, we varied the rotation speed and value of $|\ell|$ and compared the result to the predicted results (Fig. 2C). The subsidiary peaks at higher frequencies arise from a cross-coupling to different mode indices corresponding to $\Delta\ell = 37, \Delta\ell = 38$, etc. As adjacent peaks correspond to $\Delta\ell = \pm 1$, they are separated in frequency from each other by the rotation speed Ω . Most likely is that this cross-coupling arises from a slight misalignment in the experiment between the rotation and detection axes (20, 21).

The relative frequency shift between the +ve and -ve components of OAM gives rise to relative energy shifts. However, the rotation of the disk does not change the OAM spectrum of the scattered light. This can be understood as a consequence of the fact that the OAM per photon is a

discrete or quantized quantity, given by the topological charge of the vortex for the mode. This discrete quantity cannot be changed continuously, of course, and so cannot depend on the rotation frequency of the disk, which can take a continuum of possible values. Equivalently, a mode with an ℓ -fold rotational symmetry has the same symmetry when viewed in a rotating frame and hence the same OAM per photon.

The underlying mechanism introducing this frequency shift can be understood with respect to either the laboratory or the rotating frame. In the laboratory frame, the change in local ray direction, α , between the incident and detected light means that there is an azimuthal reaction force acting on the scattering surface. Doing work against this force is the energy input required to shift the frequency of the light, not dissimilar in origin to the mechanism associated with the rotation of a mode converter (22). The +ve and -ve OAM components undergo shifts in frequency (up and down, respectively), which interfere to give the modulation in intensity recorded at the detector. In the rotating frame, the incident beams are themselves seen as rotating and hence are subject to a rotational Doppler shift, with the +ve and -ve OAM components again experiencing frequency shifts up and down, respectively. Scattering centers on the surface radiate both of the frequencies back to the detector, where they again interfere to give the observed modulation in intensity.

The equivalent interpretation in terms of a Doppler shift or patterned projection applies both to OAM illumination and OAM detection. Consequently, it is possible to interchange the laser and detector. In this alternative configuration, the spinning object is illuminated directly with the expanded laser beam. Some of the scattered light is incident on the SLM, which is programmed with an identical kinoform as previously, to couple a superposition of $\pm\ell$ into the single-mode fiber. The SLM and the fiber are now acting as a mode filter to select only the desired superposition from the many modes within the scattered light. The power of light in these desired modes is a small fraction of that illuminating the object, so the light transmitted through the fiber is only of low intensity. We use a photomultiplier to measure the light transmitted through the fiber; the output is Fourier-transformed as in the previous configuration. To enhance the signal, the metal surface was lightly embossed with a pattern of 18-fold rotational symmetry, resulting in a dominant overlap with the modal superposition of $\ell = \pm 18$ ($\Delta\ell = 36$).

Figure 3 shows the frequency spectrum of the intensity modulation in the detected light as obtained over a data collection period of 5 min. Again the frequency of this peak can be predicted by Eq. 4 to reveal the rotational speed of the object.

We have shown that an OAM-based analysis of the scattered light makes it possible to infer the rotation speed of a distant object, even though the

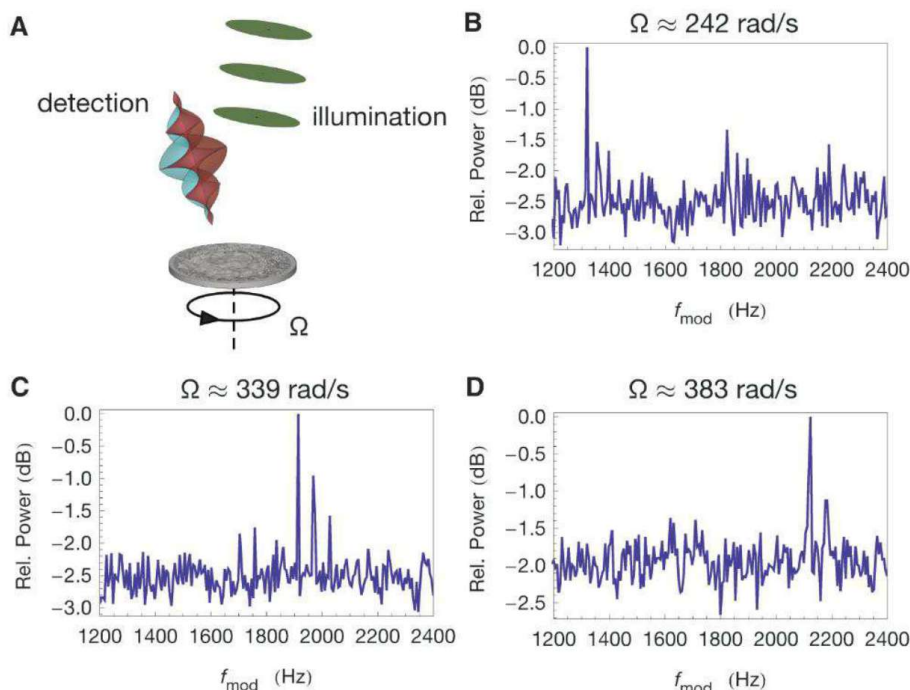


Fig. 3. Rotation rate dependence. (A) Light scattered from a uniformly illuminated spinning surface can be filtered to contain only specified modal components $\ell = \pm 18$. When these components are interfered, an intensity modulation is observed. (B to D) At rotation speeds of 242, 339, and 383 radians/s, corresponding beat frequencies of 1320 ± 20 Hz, 1914 ± 21 Hz, and 2124 ± 30 Hz were observed.

rotation axis is parallel to the observation direction. The high mode number of the OAM state, and the resulting rotational symmetry, means that the recorded frequency is higher than the rotation frequency itself by a factor of 2ℓ . A similar advantage in using OAM has been noted previously for fixed-angle measurement in both classical (23) and quantum (24) regimes. Of course, the maximum value of OAM mode that can be used is set by the modal bandwidth of the scattered light. However, increasing the bandwidth also reduces the fraction of the scattered light that falls within the scattered mode. Consequently, the degree of OAM enhancement of the rotation detection is a complicated function of the experimental conditions.

Although the Doppler shift, Doppler velocimetry, and their application to the remote measurement of transverse velocity are well known, our study recognizes that these phenomena have an angular equivalent. An analysis in terms of the OAM gives a clear and intuitive understanding of the angular case. This understanding indicates possible applications in multiple regimes. Two application areas of particular promise are the potential for the remote sensing of turbulence in

backscattered light and the possible application to astronomy for the remote detection of rotating bodies.

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Supplementary Materials

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Materials and Methods
Figs. S1 and S2
Tables S1 and S2

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Efficient Generation of H₂ by Splitting Water with an Isothermal Redox Cycle

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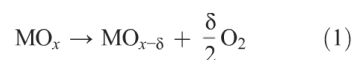
Solar thermal water-splitting (STWS) cycles have long been recognized as a desirable means of generating hydrogen gas (H₂) from water and sunlight. Two-step, metal oxide–based STWS cycles generate H₂ by sequential high-temperature reduction and water reoxidation of a metal oxide. The temperature swings between reduction and oxidation steps long thought necessary for STWS have stifled STWS's overall efficiency because of thermal and time losses that occur during the frequent heating and cooling of the metal oxide. We show that these temperature swings are unnecessary and that isothermal water splitting (ITWS) at 1350°C using the “hercynite cycle” exhibits H₂ production capacity >3 and >12 times that of hercynite and ceria, respectively, per mass of active material when reduced at 1350°C and reoxidized at 1000°C.

Hydrogen is an attractive fuel because it produces only water when burned and can be used in highly efficient fuel cells (1, 2), but it must be derived from some other chemical source, preferably a renewable one such as water (3). Splitting H₂O into H₂ and O₂ can be achieved by direct thermolysis (4–6)—for example, by solar thermal water splitting (STWS),

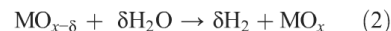
which stores solar energy as H₂ at high theoretical maximum efficiencies (7, 8). Unfortunately, direct thermolysis requires temperatures exceeding 2700°C that are impractical for industrial pro-

cesses (9). However, two-step thermochemical H₂O splitting based on metal oxide reduction and oxidation (redox) cycles produces appreciable amounts of H₂ at the more technically feasible reduction temperatures T_{red} of 1200° to 1500°C (4, 10).

In traditional two-step temperature-swing water splitting (TSWS), O₂ is generated by the reduction of a metal oxide during a high-temperature step according to



where $T_{\text{red}} \approx 1200^\circ$ to 1500°C . The second step involves lowering the temperature and exposing the reduced metal oxide to water, which reoxidizes the metal oxide and produces H₂ according to



where oxidation temperature $T_{\text{ox}} < T_{\text{red}}$. One major advantage of this approach is that although the complete cycle produces H₂ and O₂ from H₂O,

Table 1. H₂ production capacities and peak rates. A comparison of the solar thermal water splitting cycle capabilities is shown for both temperature swing and isothermal operation.

Operating temperature conditions (Red/Ox, °C)	Total H ₂ generated (μmol g ⁻¹)	Peak H ₂ generation rate (μmol g ⁻¹ s ⁻¹)
Hercynite 1350/1000	31.4 ± 2.3	0.06 ± 0.04
Hercynite 1500/1200	93.7 ± 19.2	0.32 ± 0.01
Hercynite 1350/1350	102 ± 18	0.55 ± 0.16
Ceria 1350/1000	16.4 ± 3.6	0.14 ± 0.04

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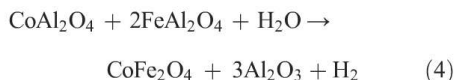
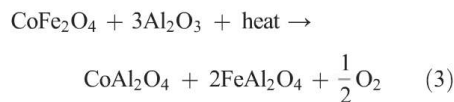
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they are produced in two separate steps, thus simplifying separation. Traditional thermodynamic analysis treats the process as a closed system and suggests that a substantial temperature difference between the oxidation and reduction steps is necessary to split water (10–13). However, large temperature swings between the reduction and oxidation steps cause thermodynamic inefficiencies from the irreversible heat losses incurred upon cooling of the active material to the oxidation temperature and the heat required solely to reheat the active material to its reduction temperature. Additionally, thermal stresses arising from the rapid thermal cycling of the system over large temperature differences present engineering and materials challenges in the design of high-temperature redox systems. However, contrary to widely accepted re-

dox gas-splitting theory (11), we demonstrate that a change in temperature between reduction and oxidation steps is unnecessary, and that isothermal water splitting (ITWS) driven by swings in steam partial pressure not only produces H_2 , but outperforms traditional TSWS and provides an opportunity for expanding renewable H_2 generation.

We used the “hercynite cycle” to examine TSWS and ITWS. Unlike more conventional non-volatile metal oxide redox chemistries, in which reduction results in either O vacancy formation (e.g., cycles involving fluorite- or perovskite-type crystal structures such as CeO_2) (14) or the formation of solid solutions (e.g., ferrites) (15–18), reduction occurs via reaction between MFe_2O_4 and Al_2O_3 to form stable aluminates (19, 20). During oxidation by H_2O , the M-ferrite spinel

(MFe_2O_4) and alumina (Al_2O_3) reform, liberating H_2 . For cobalt, the governing reactions are



Reduction via reaction 3 begins at temperatures as low as 940°C, ~150°C below where ferrites and ceria start to reduce because the formation of the stable aluminates is more thermodynamically favorable than the formation of solid solutions or vacancies. Hence, the hercynite cycle is selected to demonstrate ITWS.

We used a hercynite cycle–active material composed of 19.8 weight percent $CoFe_2O_4$ on Al_2O_3 (21, 22) in a stagnation flow reactor (23) to establish the baseline for comparison of TSWS to ITWS, as outlined in the supplementary materials and shown in fig. S1. In TSWS, the active materials are reduced (reaction 3) under inert gas flow (He) at 1350°C and 101.3 kPa for 60 min, then cooled to 1000°C for water reoxidation (reaction 4). A gas stream of 50 volume percent (vol%) steam in inert He was used to oxidize the reduced materials for 25 min, producing $31.4 \pm 2.3 \mu\text{mol}$ of H_2 per gram of total material (Table 1). The extent of reduction and water-splitting capacity depends on the reduction temperature. Higher temperatures lead to a larger fraction of reduced Fe^{2+} ions. The number of available Fe^{2+} ions dictates the total H_2 -generating capacity of the active material, with a maximum ratio of one H_2 molecule produced to every two Fe^{2+} cations. In our experiment, oxidation at 1000°C was characterized by a slow rate of reaction (a peak H_2 generation rate of $0.06 \pm 0.04 \mu\text{mol g}^{-1} \text{s}^{-1}$) and was likely surface reaction–limited (20). Because the oxidation reaction is slow, it is unlikely that most of the Fe^{2+} ions are reoxidized to Fe^{3+} during the 25 min, which limits the total H_2 production capacity of the 1350°/1000°C TSWS cycle.

Higher TSWS reduction and oxidation temperatures result in increased H_2 and O_2 production capacity and water-splitting rates. Active material cycled between 1500° and 1200°C produced $93.7 \pm 19.2 \mu\text{mol } H_2 \text{ g}^{-1}$ with a peak rate of $0.32 \mu\text{mol } H_2 \text{ g}^{-1} \text{s}^{-1}$ (Table 1). The higher reduction temperature resulted in more complete reduction of the active material and a larger thermodynamic driving force for H_2O splitting. The higher rates of H_2 generation at 1500°/1200°C resulted from two factors: (i) the intrinsically higher reaction rates that arise from carrying out a kinetically limited process at a higher temperature, and (ii) the larger thermodynamic driving force for oxidation, which comes from the larger extent of reduction. The ability to perform water oxidation at temperatures above the onset of reduction suggests that ITWS is possible (24) and that the current

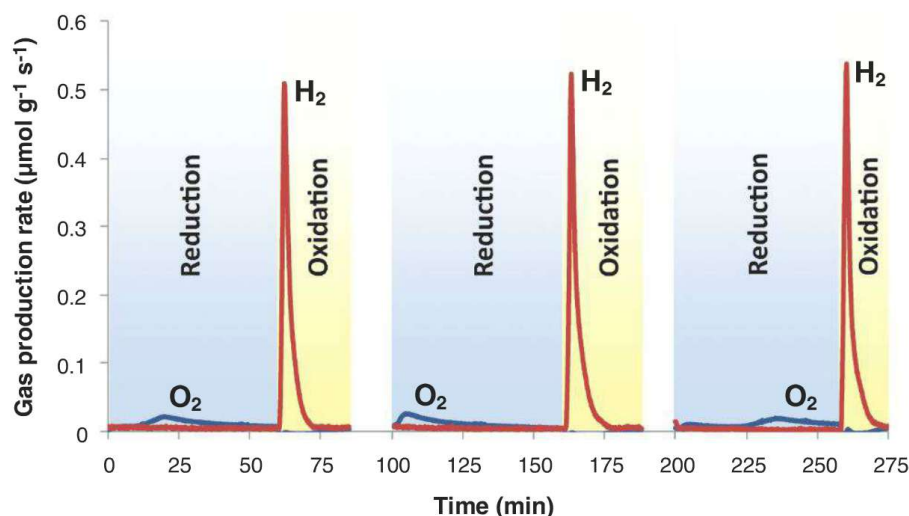


Fig. 1. Isothermal water splitting at 1350°C. The H_2 and O_2 generation rates are shown in red and blue, respectively.

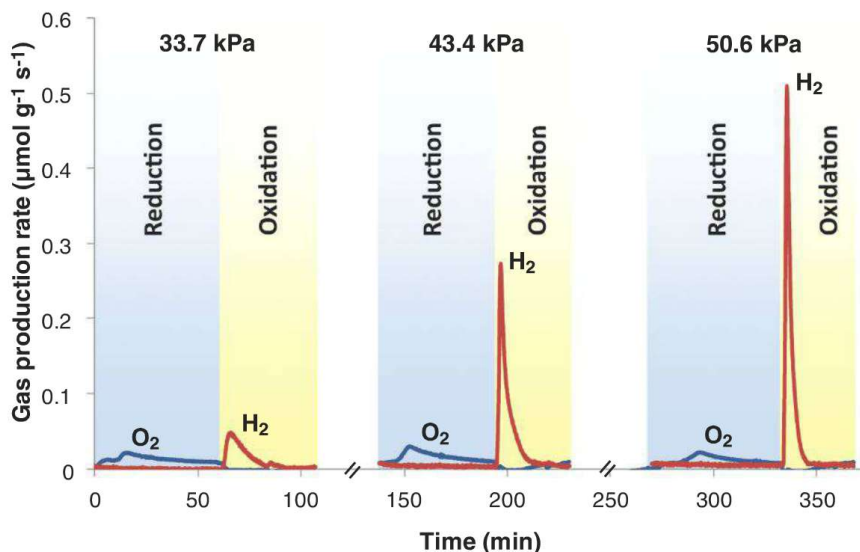


Fig. 2. The effect of steam pressure on 1350°C ITWS. From left to right: H_2O partial pressures of 33.7 kPa, 43.4 kPa, and 50.6 kPa.

understanding of the thermodynamics of STWS is incomplete.

Hercynite cycle-active materials split water in a two-step isothermal redox cycle, as exemplified by the 1350°C ITWS results shown in Fig. 1. H₂ generation under ITWS conditions was achieved at a temperature above the minimum reduction temperature by first flowing an inert gas through the reactor to sweep away O₂ generated during the reduction step and subsequently injecting steam during the oxidation step to produce H₂ (fig. S3). For 1350°C ITWS, the active particles produced $45.1 \pm 7.6 \mu\text{mol O}_2 \text{ g}^{-1}$ during 1 hour of reduction and $102 \pm 18 \mu\text{mol H}_2 \text{ g}^{-1}$ during 25 min of exposure to 50 vol% steam in He. During reduction, the initial O₂ plateaus arise from incomplete H₂O removal from the system. Once all steam is swept from the reactor, the O₂ peak occurs as the materials reduce, followed by the normal exponential decay as the reaction goes to completion. 1350°C ITWS produces substantially more O₂ and H₂ than 1350°/1000°C TSWS and slightly more O₂ and H₂ than 1500°/1200°C TSWS. The slight deviation of the 2.26:1 H₂/O₂ ratio from the expected 2:1 ratio, although within error, likely arises from slight differences in the sensitivities and response times of the O₂ analyzer and of the mass spectrometer used for measuring H₂. The higher gas-splitting production capacity of ITWS was accompanied by a higher H₂ production rate of $0.55 \pm 0.16 \mu\text{mol H}_2 \text{ g}^{-1} \text{ s}^{-1}$. ITWS's higher rate of H₂ generation and its lack of irreversible heat losses associated with the change in temperature between oxidation and reduction steps (which is required for TSWS) results in ITWS having a higher theoretical efficiency than TSWS, with the conditions examined in this study (see the supplementary materials for a comparison of ITWS and TSWS efficiencies, in particular table S1).

Traditional TSWS thermodynamic theory uses a closed-system model in which an oxidation temperature lower than the reduction temperature raises the chemical potential of the oxidizing gas and reduced metal oxide above that of the product gases and oxidized material in order to drive the oxidation reaction. In contrast, ITWS in an open system uses swings in the partial pressure of the oxidizing gas to produce the chemical potential

differences that drive the oxidation and reduction steps. To form a two-step water-splitting closed-cycle system, a process for the recombination of H₂ and O₂ must be included, such as an electrolyzer (fig. S4) (5). In the ITWS process, the partial pressure of generated gaseous reduction and oxidation products remains low by sweeping them from the reactor. The low chemical potential of O₂ during reduction and high H₂O and low H₂ chemical potentials during oxidation drive the respective reactions forward to enable ITWS. Furthermore, sweeping the product gases away from the metal oxide prevents reverse reactions from occurring.

Not only are redox reactors open systems, but the reactions take place between gases adsorbed on the surface and the solid phase. Thus, the chemical potential and the coverage of gas molecules adsorbed on the surface are the relevant quantities to consider for analyzing redox reaction thermodynamics. Because the coverage of adsorbates, and therefore their chemical potentials, are related to partial pressure, the oxidation reaction is driven by increasing the H₂O partial pressure and maintaining a low H₂ partial pressure. Therefore, ITWS relies on a chemical potential difference derived from readily adjustable gas-phase partial pressures, rather than having to depend on the problematic large temperature changes of TSWS.

This analysis suggests that by increasing the partial pressure of the oxidizing gas, we can drive the water-splitting reaction further toward the products. Indeed, an increased partial pressure of water resulted in higher H₂ production capacities at higher rates (Fig. 2). For 1350°C ITWS, steam concentrations in He of 33%, 43%, and 50% (overall pressure held at 101.3 kPa) correspond to H₂ production capacities of 40 ± 9 , 72 ± 8 , and $102 \pm 18 \mu\text{mol H}_2 \text{ g}^{-1}$ and peak production rates of 0.06 ± 0.02 , 0.15 ± 0.07 , and $0.55 \pm 0.16 \mu\text{mol H}_2 \text{ g}^{-1} \text{ s}^{-1}$, respectively. The increased peak production rates correspond to shorter oxidation times, as expected. The higher steam pressure results in a higher water chemical potential on the active material's surface, which provides a higher thermodynamic driving force for oxidation. Additionally, the higher steam concentrations increase the overall rate

of reaction by increasing the reactant concentration. This result suggests that decreases in the time required for the oxidation step and possible increases in the ITWS temperature can be achieved by increasing the partial pressure of the oxidizing gas. Furthermore, relative to the 1350°/1000°C ceria redox cycle (which is considered the current state-of-the-art material for TSWS redox processing), the ITWS hercynite cycle produces >6 times as much H₂ on a total-mass basis and >12 times as much H₂ on an active-materials basis (47% active) (Fig. 3 and Table 1). In addition to ITWS's favorable kinetics and thermodynamics (Fig. 3 and table S1), ITWS reduces both irreversible heat losses and thermal shock concerns that limit the efficiency and operations of traditional TSWS.

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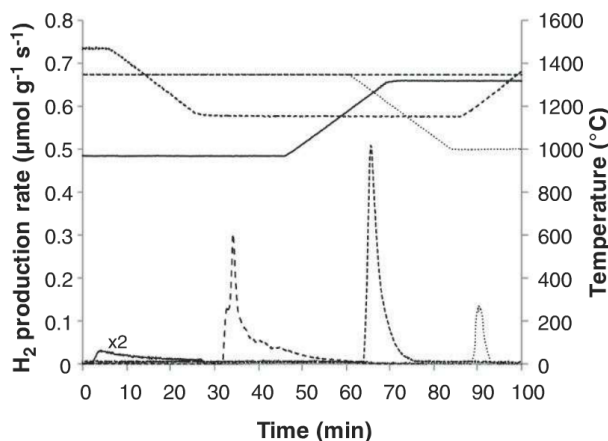
Supplementary Materials

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Figs. S1 to S4
Table S1
Reference (25)

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Fig. 3. A comparison of STWS.

H₂ production curves are shown for hercynite cycle-based 1350°/1000°C TSWS (solid line, shown at twice its experimentally found value), 1500°/1200°C TSWS (long dashed line), 1350°C ITWS (short dashed line), and ceria-based 1350°/1000°C TSWS (dotted line).



Anthropogenic Seismicity Rates and Operational Parameters at the Salton Sea Geothermal Field

Emily E. Brodsky* and Lia J. Lajoie

Geothermal power is a growing energy source; however, efforts to increase production are tempered by concern over induced earthquakes. Although increased seismicity commonly accompanies geothermal production, induced earthquake rate cannot currently be forecast on the basis of fluid injection volumes or any other operational parameters. We show that at the Salton Sea Geothermal Field, the total volume of fluid extracted or injected tracks the long-term evolution of seismicity. After correcting for the aftershock rate, the net fluid volume (extracted-injected) provides the best correlation with seismicity in recent years. We model the background earthquake rate with a linear combination of injection and net production rates that allows us to track the secular development of the field as the number of earthquakes per fluid volume injected decreases over time.

The exploitation of geothermal resources is rapidly expanding as society increases its reliance on renewable energy sources. Production of geothermal power induces seismicity as water is pumped both into and out of a reservoir (1, 2). Fluid injection, a major component of most geothermal operations, has been shown to induce seismicity in a variety of settings, and the earthquakes are often attributed to a decrease in the effective stress on faults due to increased pore pressure (3–7). Earthquakes can also be induced by fluid extraction through more complex processes, such as thermal contraction or subsidence-driven increases in shear stress (8, 9). Seismic consequences of geothermal production are of particular concern for facilities neighboring large tectonic systems. Therefore, it is important to understand the controls on geothermal production-related seismicity in a setting such as the Salton Sea Geothermal Field, which borders the diffuse terminus of the San Andreas Fault system in southern California.

The Salton Trough is home to four operating geothermal fields (Salton Sea, Brawley, Heber, and East Mesa) that generate a total of over 650 MW of electricity (Fig. 1). The Salton Sea Geothermal Field exploits a hot, geothermal brine, with temperatures in excess of 320°C at 2 km depth (10, 11). The field includes 10 operating geothermal power plants. Plants use flash technologies that extract fluid from depth and flash a portion to steam in order to power generators, while the remaining brine is either injected via separate wells back into the reservoir or subjected to further flashing. Steam condensate is also recaptured for injection. The volume of fluid loss during operations depends on a variety of conditions, including salinity in the flashing chambers and air temperature. Since 1992, the

average reported monthly injection volume is 81% of the reported production volume, with a standard deviation of 7%. Production at the plant cycles annually in response to demand and local environmental conditions.

The first plant came online at the Salton Sea Geothermal Field in 1982 (10). Operations expanded steadily from 1991, with new wells regularly added and fluid volumes increasing through 2005 (Figs. 1B and 2). For the highest production month in 2012, 11.2×10^9 kg ($\sim 10^7$ cubic meters) of geothermal fluid was extracted from the reservoir at depths of ~ 1 to 2.5 km, and 9.2×10^9 kg was injected at similar depths (12, 13).

We quantified the relationship between fluid volumes within the Salton Sea Geothermal Field and the local rate of seismicity by combining publicly available data sets. By law, in California the monthly fieldwide total production and injection volumes are released to the California Department of Conservation. For earthquake locations and times, we used the largest high-precision catalog available for the region (14) for January 1981 to June 2011 supplemented by the Southern California Seismic Network catalog for July 2011 to December 2012. Station configurations have changed over time, with a notable increase in data after the release of the geothermal field's local seismic network in 2007. Therefore, we restricted the data to the local magnitude of completeness of 1.75 (supplementary text) to ensure that we only analyze events that are large enough to have been detectable throughout the study period. For this study, the Salton Sea Geothermal Field seismicity is defined as earthquakes shallower than 15 km in the region bounded by 33.1 to 33.25° N and 115.7 to 115.45° W (Fig. 1).

The seismicity rate has mirrored overall activity in the field (Fig. 1 and fig. S2). As noted by (15), earthquakes cluster around injection wells both at the surface and at depth. The seismicity rate was initially low during the period of low-

level geothermal operations before 1986. As operations expanded, so did the seismicity. The maximum net volume production rate occurred in July 2005, which is a month before the largest earthquake rate increase. This August 2005 swarm has been linked to a creep transient but had not been compared previously with the production data (16, 17).

However, the relationship between seismicity and plant operations is not simple. Earthquakes are highly clustered owing to local aftershock sequences, and so it is difficult to untangle the direct influence of human activities from secondary earthquake triggering. In addition, seismicity rate varies over orders of magnitude, whereas pumping conditions evolve more smoothly. Operations are continually changing at the plants in response to both economic and natural factors. These issues complicate the data so that a simple correlation between the raw seismicity rate and operational parameters is suggested but unclear and requires further analysis (fig. S2).

Because earthquakes commonly have aftershocks, any statistical method that assumes independence of events is problematic. A better approach is to measure the background rate over time, separate from the secondary triggering of aftershocks. In this context, the term “background rate” means the primary earthquakes directly related to the driving stress from both tectonic and anthropogenic sources, and therefore the background rate can vary in time.

To separate background and aftershock seismicity, we used the Epidemic Type Aftershock Sequence (ETAS) model, in which background and aftershock rates are parameterized by using standard empirical relationships, and the resulting parameter set is simultaneously fit from the observed catalog (18). This strategy builds on previous work on identifying fluid-modulated signals in natural and induced seismicity (18–21). The seismicity is modeled as a Poissonian process with history-dependent rate R_{ETAS} at time t_E that is a combination of the modified Omori's law, which describes the temporal decay of aftershocks; the Gutenberg-Richter relationship, which describes the magnitude distribution; and the aftershock productivity relationship

$$R_{\text{ETAS}}(t_E) = \mu + \sum_{i: t_i < t_E} \frac{K_E 10^{\alpha(M_i - M_c)}}{(t_E - t_i + c)^p} \quad (1)$$

where μ is background seismicity rate, and the term inside the summation describes the component of seismicity due to aftershock sequences. In this formulation, K_E is the aftershock productivity of a mainshock, α describes the efficiency of earthquakes of a given magnitude at generating aftershocks, c and p are Omori's law decay parameters, M_i and t_i are the magnitude and time of the i^{th} event in the catalog, and M_c is the magnitude completeness threshold of the catalog (supplementary materials, materials and methods) (18, 22).

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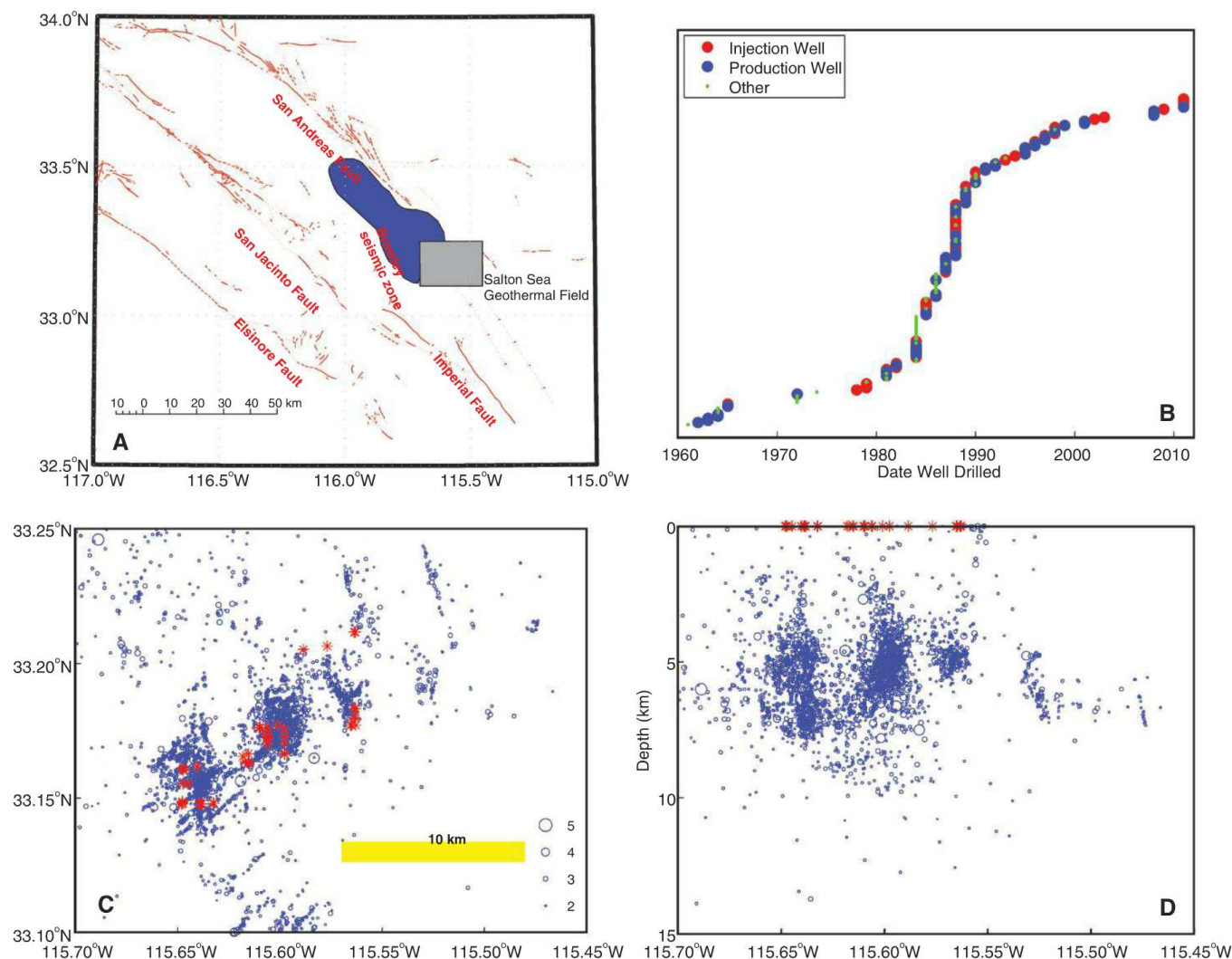


Fig. 1. Earthquake and geothermal facility locations and activity. (A) Regional map with faults and location of the Salton Sea Geothermal Field. (B) Drill year of the wells in the field for 1960–2012. (C) Earthquakes (blue circles) and injection wells (red stars) in map view.

(D) East-west cross-sectional view. Earthquake hypocenters cluster around and beneath injection wells. Depth is relatively poorly constrained in the sedimentary basin (27) and is not used for any subsequent statistics in this study.

We performed maximum likelihood fits on overlapping 2-year windows and tracked background seismicity rate over time (Fig. 2 and fig. S3). We assumed that the background rate is stationary for relatively short times—that is, over the 2-year interval—but varies over the long duration of the full catalog. The 2-year interval allows sufficient events to have well-resolved parameters (fig. S4), while still capturing the high-frequency fluctuations (fig. S5).

The increasing trend of the background rate μ in Fig. 2 imitates all the metrics of fluid volume (injection, production, and net production, defined here as production minus injection), particularly in the earlier years of injection (until ~1991). In later years (2006 to 2012), μ tracks net production more closely (Table 1). In between, seismicity may track net production with a baseline shift (Fig. 2C), but the correlations are much less clear. Both total and net production seem important.

The time variable behavior is captured by the best-fit coefficients of a linear model

$$M = c_1 I + c_2 N \quad (2)$$

where I and N are the injection and net production rate, respectively, and c_1 and c_2 are the coefficients. Because the correlation between total injection and total production is extremely high, only one of those variables is used in Eq. 2 so as to ensure a well-constrained solution. We measured time variation by fitting Eq. 2 over a moving data window that is longer in duration than the window used to fit the ETAS model and much shorter than the full study interval. A linear least-squares fit in a 6-year window with 0.5-year increments (~90% overlap) captures the essential features (Fig. 3).

The combined model of Eq. 2 results in well-constrained model parameters after the initial growth of operations in the mid-1980s.

An F test rejects the null hypothesis of insignificant fit at the 95% level for all time periods except during the rapid growth of the field in 1993. The early years have substantial uncertainty in the fit coefficients, as might be expected during the highly nonsteady initial phase of operations. During the well-fit period, the seismicity rate generated per monthly volume of fluid injected steadily decreases over time. Net production generated more earthquakes per fluid volume than did injection both early and late in the study period.

In addition to the difference in correlations over time, there is a difference in phase lags between seismicity and the various operational parameters. The phase lag corresponding to the maximum correlation of seismicity relative to net production is usually 0 months (Table 1). However, the intervals with the strongest correlations between injection or production and seismicity can have several-month phase lags.

Fig. 2. Background seismicity rate (μ) over time compared with operational fluid volumes at the Salton Sea Geothermal Field. The seismicity rate curve is identical for each panel (right axis, green curve), and the operational rate (left axis, blue curve) in each case is (A) production rate, (B) injection rate, and (C) net production rate. Seismicity rate estimation is on 2-year overlapping intervals centered on each month for which there is operational data. Confidence levels on μ are mapped in fig. S3.

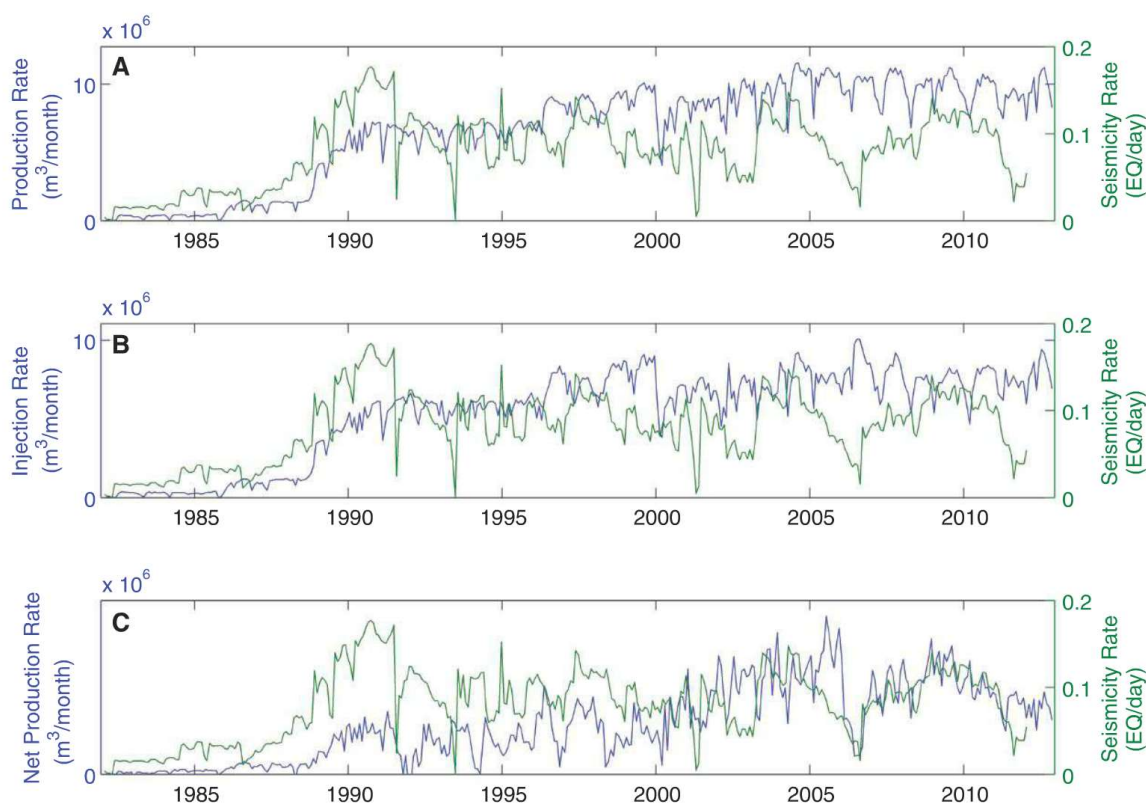


Table 1. Cross-correlation of operational parameters with seismicity rate. Reported values are maxima of the normalized cross-correlation between background seismicity μ reported in Fig. 3 and the given operational rate, with means removed. Normalization is by the geometric mean of the autocorrelations. Lags are time shifts corresponding to the max-

imum cross-correlation and are reported in months. Lags are restricted to positive (seismicity lagging behind operation) so as to limit cases to only physical scenarios. Time series run from 1 January to 1 January of the reported years. Alternative model assumption results are in table S1 (supplementary text).

Fluid metric	1982–2012			1982–1991			1991–2006			2006–2012		
	Max cross-correlation	Lag		Max cross-correlation	Lag		Max cross-correlation	Lag		Max cross-correlation	Lag	
Production volume	0.61	7		0.95	0		0.21	160		0.15	59	
Injection volume	0.64	8		0.96	6		0.26	89		0.14	45	
Net production volume	0.47	2		0.92	0		0.18	170		0.69	0	

Over the full data set, the maximum correlation between seismicity and injection has a lag of 8 months (Table 1). In intervals such as 1991–2006, unphysical, large phase lags accompany low correlations and are another indicator of the lack of predictive power of a single operational parameter during these periods.

We conclude from the observed correlations and the F test that net production volume combined with injection information is a good predictor of the seismic response in the short term for a fully developed field. Much previous work (3, 5–7, 23) has focused on the increase in pore pressure (decrease in effective stress) from injection as the primary driver of seismicity, and the importance of net production (volume removed) suggests that seismicity is responding to a more complex process. Earthquakes responding to net volume loss with no phase lag may imply that seismicity responds to elastic compaction and subsidence and not

simply diffusion of high pore pressures at injection sites (8, 9).

An important issue for any induced seismicity study is the possibility of triggering a damaging earthquake. Like most earthquake sequences, the Salton Sea Geothermal Field seismicity is dominated by small earthquakes, and the magnitude distribution follows the Gutenberg-Richter relationship: The number of earthquakes of magnitude greater than or equal to M is proportional to 10^{-bM} , where b is nearly 1 (the maximum likelihood estimate of b for 1982–2012 is 0.99) (fig. S6). Static and dynamic stresses have been observed to trigger earthquakes on disconnected fault networks (24, 25), and the Gutenberg-Richter relationship generally holds for the aggregated sequences (18, 26). The major limitation in applying Gutenberg-Richter in a particular region is estimating the maximum size of an earthquake that can be hosted by the local faults. The largest earthquake in the Salton Sea Geothermal field region during the study

period was of 5.1 magnitude, and the neighboring, highly strained San Andreas fault can have earthquakes of magnitude at least 8.

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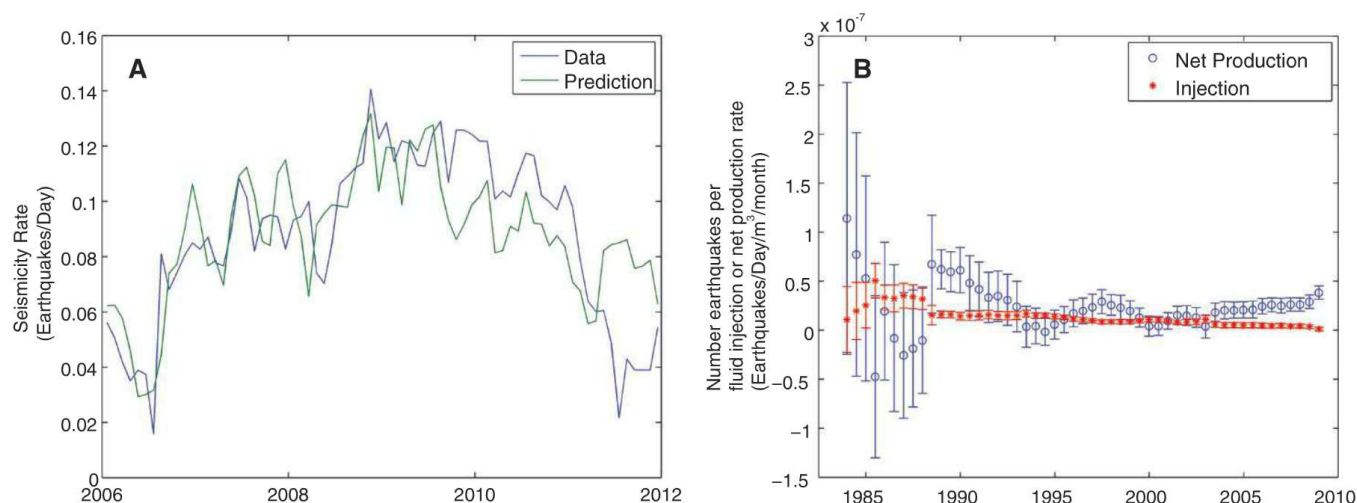


Figure 3. Results of linear model of seismicity based on a combination of injection and net production. (A) Sample seismicity rate and model prediction of seismicity rate using the observed fluid data and the best-fit linear model of Eq. 2. **(B)** Number of earthquakes per day trig-

gered per rate of net volume of fluid extracted or total fluid injection. Symbols are best-fit coefficients for Eq. 2. The injection values are coefficient c_1 in Eq. 2, and net production values are c_2 . Error bars are 2 SD of model estimates based on the linear regression.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1239213/DC1
Methods
Figs. S1 to S7
Table S1
References (28–36)

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Two Dimensions of Value: Dopamine Neurons Represent Reward But Not Aversiveness

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Whereas reward (appetiteness) and aversiveness (punishment) have been distinguished as two discrete dimensions within psychology and behavior, physiological and computational models of their neural representation have treated them as opposite sides of a single continuous dimension of “value.” Here, I show that although dopamine neurons of the primate ventral midbrain are activated by evidence for reward and suppressed by evidence against reward, they are insensitive to aversiveness. This indicates that reward and aversiveness are represented independently as two dimensions, even by neurons that are closely related to motor function. Because theory and experiment support the existence of opponent neural representations for value, the present results imply four types of value-sensitive neurons corresponding to reward-ON (dopamine), reward-OFF, aversive-ON, and aversive-OFF.

In our common use of language, we typically treat “reward” and “punishment” as two qualitatively discrete categories. Many sensory

stimuli can be readily classified as either appetitive or aversive, and we distinguish between a less-than-expected punishment and a greater-than-

expected reward. Likewise, reward and punishment have often been considered as two distinct dimensions within the study of psychology and behavior, with appetitive and aversive stimuli eliciting approach and avoidance behaviors, respectively (*1*). If they constitute two distinct categories, then reward and punishment are not opposites of one another. However, to decide on motor outputs, the brain must effectively evaluate actions on a common scale in which evidence of good is counterbalanced by evidence of bad. Simple and elegant models have been based on neurons that represent both good and bad along a single continuum of value, analogous to light and dark on the single dimension of light intensity. Most previous work on the physiology and computational function of dopamine (*2–10*) and other value-sensitive neurons (*11–15*) has proposed,

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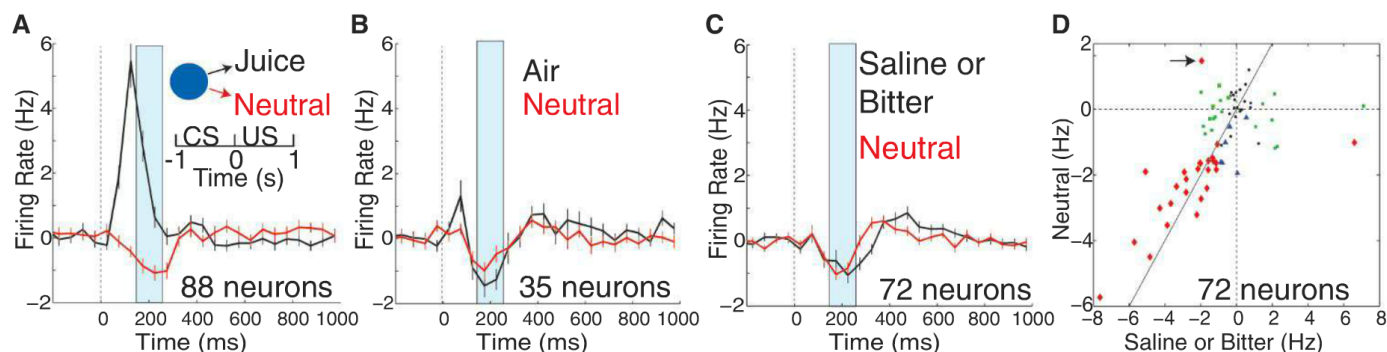


Fig. 1. Dopamine neurons are not activated by omission of an expected aversive stimulus. Monkeys were conditioned with audiovisual Pavlovian stimuli to expect a stimulus (after a 1.0-s delay) that was either neutral sound or had appetitive or aversive value [(A) inset and fig. S1A]. (A) Juice (black) and its absence (red) caused an increase and decrease in average firing rate, respectively, across a population of 88 neurons. Neuronal discrimination of value was best at 150 to 250 ms after stimulus onset (shaded region) (16). All peri-stimulus time histograms (bin size, 50 ms) are averages across all recorded neurons, some of which were unresponsive. (B) Both air (black) and its absence (red) caused suppression. Unlike (A), (C), and (D), data are only from monkey F.

(C) Both saline or bitter (black) and its absence (red) caused suppression. (D) Firing rates (150 to 250 ms, baseline rates subtracted) of each neuron to saline (or bitter) and neutral outcomes. The arrow indicates a single neuron in which the neutral stimulus caused activation, which is consistent with the single-dimension hypothesis. Symbols indicate results of *t* tests: activation or suppression to saline-bitter (green squares), to the neutral stimulus (blue triangles), both (red diamonds), or neither (black circles). The diagonal line indicates identity. Pearson's correlation $r = 0.63$; $P < 10^{-8}$. Of these 72 neurons, 8, 2, and 62 were from the ventral tegmental area, retrorubral field, and substantia nigra, respectively; 35 were from the dorsal tier, and 37 were from the ventral tier.

explicitly or implicitly, that neurons represent “total value” along a single dimension.

Prior studies supported the proposition that dopamine neurons are activated or suppressed by anything that is better or worse than expected, respectively (2, 8). They have been proposed to signal a “reward prediction error” that drives reinforcement learning, teaching dopamine-recipient neurons both what is good and what is bad (2, 9). One would expect that if dopamine represents both reward and aversiveness on a single dimension of total value, so too may dopamine-recipient neurons throughout much of the brain. However, it has not been shown that either dopamine or any other reward-sensitive neuron is also sensitive to aversiveness, as required by the “single-dimension” hypothesis. The alternative “two dimensions” hypothesis is that such neurons are sensitive only to reward, and that other neurons should be sensitive to only aversiveness.

Testing these alternatives is more challenging than it may initially appear. First, neuronal responses are not necessarily related to motivational value. Short latency activation (<100 ms) of dopamine neurons by aversive air puff is related to its high sensory intensity, not its aversiveness (16, 17). This sensory-related activation is to be expected of any neuron that represents value in a general manner (16), and it appears to have been misattributed to aversiveness in at least one study (6), as shown previously (17). Second, to characterize any single neuron both appetitive and aversive stimuli must be presented in temporal proximity to one another. This creates challenges because if a stimulus is overly aversive, it will interfere with performance of an appetitive task. The aversive stimulus must therefore be mild (such as an air puff to the face), and a low cost-avoidance response (such as eye blink) does not insure a net aversive value (as in

the case of blinking in response to a cool breeze on a hot day). It is questionable whether the stimuli tested in some previous studies did in fact have net aversive value. Third, we need to estimate the subjective value of aversive stimuli (aversiveness) on a common scale with subjective reward value and to then compare neuronal responses to stimuli of approximately equal but opposite values. If aversiveness is too low it may be ineffective in modulating neurons, especially if it is overshadowed in the context of a reward stimulus with much greater absolute value [as expected given principles of predictive (optimal) coding exemplified by dopamine neurons (18)]. Studies that have examined responses to both appetitive and aversive stimuli in the same neurons have generally not addressed these issues and are thus inconclusive with respect to the present hypotheses (4, 6, 11–15, 19–21). None of the past studies discussed the possibility that reward and aversiveness could be two discrete dimensions to be represented by discrete neurons.

Data are from electrophysiological single-unit recordings of 195 dopamine neurons in two rhesus macaques (22, 23). Previous analyses of this same data set characterized the multiphasic temporal dynamics of neuronal responses, as well as their dependence on anatomical location within the ventral midbrain, among other issues (16, 17, 24). A critical and distinguishing feature of these experiments was the use of a choice task to quantify how much juice reward a monkey would sacrifice in order to avoid an aversive stimulus (air puff to the nose or oral delivery of saline or bitter solution). The subjective value of each stimulus was repeatedly measured and adjusted in intensity until it was eventually fixed to have an aversiveness comparable with a typical drop of juice (130 μ l), with average values of -70 to -110 μ l for each stimulus (except -200 μ l in the

case of concentrated bitter solution) (16). The aversiveness of the air puff was at least an order of magnitude greater than that necessary to elicit conditioned eye blink, and it is thus likely to have been much greater than that used in previous studies (16). Neurons were not recorded during the choice task, but in simple Pavlovian tasks that used identical aversive stimuli (fig. S1). Eye position was measured, and gaze toward or away from Pavlovian conditioned stimuli demonstrated that monkeys had learned to expect the appetitive or aversive outcomes, respectively (fig. S2).

In accord with the single-dimension hypothesis, it is well known that aversive stimuli suppress the firing of dopamine neurons. However, that hypothesis also proposes that aversive or neutral stimuli that are not as bad as (“better than”) expected should cause activation. Given a simple and well-established experimental design, we can be very precise about the amplitude of the activation. When a Pavlovian conditioned stimulus (CS) (or instrumental action) predicts subsequent reward or no reward with equal probability, reward delivery causes strong activation, and its omission causes suppression of firing rate, as shown here (Fig. 1A) and elsewhere (6, 18, 22). In fact, the amplitude of this activation was previously found not to depend on the value of the reward, at least over a range of 50 to 500 μ l of juice (this may appear strange, but it is evidence of optimally efficient coding) (18). All of the aversive stimuli studied here had absolute values greater than 50 μ l. Therefore, the single-dimension hypothesis makes the strong prediction that the omission of any of the aversive stimuli in this task will cause virtually the same activation as the delivery of juice reward shown in Fig. 1A.

However, no activation was observed (Fig. 1, B to D). Of 72 neurons tested with saline or bitter,

only one was significantly activated by the neutral outcome, whereas 40 and 49% (29 and 35 neurons) were significantly suppressed by the neutral and aversive outcomes, respectively, and 32% (23 neurons) were suppressed by both (Fig. 1D) ($P < 0.05$, unpaired t tests). Even among 12 neurons tested with a high concentration of bitter having an aversiveness of -0.2 ml of juice (a greater absolute value than that of the juice in Fig. 2A and in most primate studies), six were significantly suppressed by omission of bitter, and none were activated. Similarly, not one of 35 neurons was significantly activated by omission of air, whereas 11 were significantly suppressed. The lack of activation to a neutral outcome was not due to a lack of sensory stimulation because omission of the aversive stimulus was signaled by the onset of a distinct sound (72 dB, similar intensity to the sound caused by the opening of valves that deliver juice, saline, and bitter solutions).

The single-dimension hypothesis implies that dopamine neurons should signal prediction errors for aversiveness in the same manner that

they do for appetitiveness. However, whereas reward stimuli only caused substantial activation when they are unpredicted (Fig. 2A), prediction had at most a marginal effect on suppression by aversive stimuli (Fig. 2, B to D). Across the population of neurons, there was no significant difference between responses to predicted versus unpredicted air or saline-bitter ($P > 0.5$ for each stimulus in each monkey; paired t tests across 30 to 47 neurons in each of the four groups). Among 67 neurons, 11 had significantly higher firing rates (less suppression) to predicted versus unpredicted saline or bitter, and eight had the opposite relationship (Fig. 2D). Similarly, 15 and 7 of 77 neurons had higher firing rates for predicted and unpredicted air, respectively.

These data clearly contradict the single-dimension hypothesis but can be explained if reward and aversiveness are represented as two dimensions. It is proposed that dopamine neurons add together evidence for (excitation) and against ("opponent" inhibition) reward (16) but are not directly influenced by aversiveness. In

this view, aversive and neutral stimuli suppress firing because they provide evidence against reward. Stimuli explicitly conditioned to predict absence of reward have been shown to suppress activation of dopamine neurons (25). The aversive and neutral stimuli studied here became familiar through conditioning, and they predicted an absence of reward within a general context that was associated with reward (indeed, there is a chance of reward in any context).

As a final test, aversive stimuli were delivered together with juice. The single-dimension hypothesis states that the only important factor is net value, the sum of reward and aversive values. The two-dimensions hypothesis predicts that a purely aversive stimulus will affect dopamine neurons only if it alters the value of a reward stimulus. Because concentrated saline and bitter solutions were delivered into the mouth together with juice, they would be expected to strongly devalue it. In contrast, simultaneous delivery of air to the nose would be expected to have little interaction with juice and thus should be less effective in devaluing it.

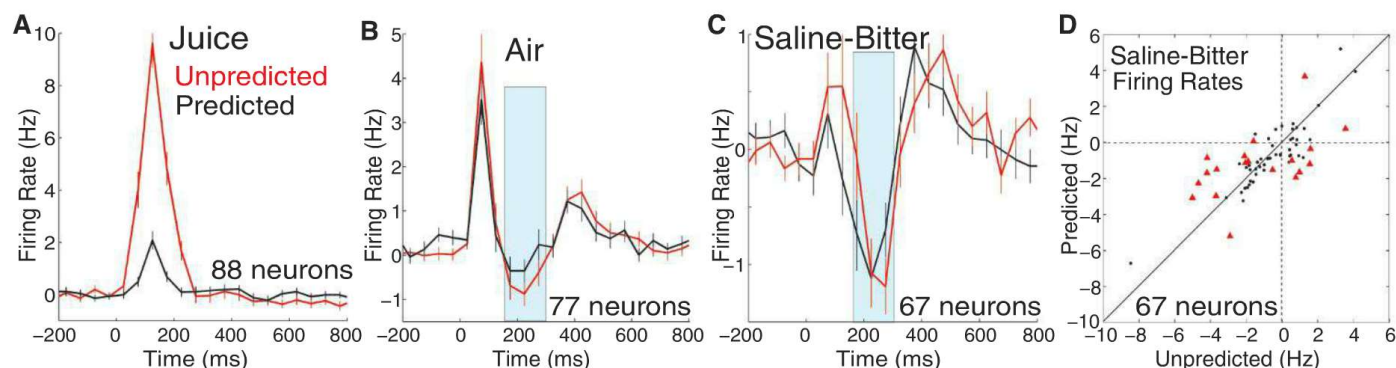


Fig. 2. Suppression by aversive stimuli is insensitive to prediction. Predicted stimuli occurred 1.0 s after a CS, whereas "unpredicted" stimuli were delivered once every 2 to 16 s with no CS (fig. S1B). There are differences in scales of y axes across (A) to (D). (A) Unpredicted (red) but not predicted (black) juice reward caused strong activation. (B) Prediction only marginally diminished the sensory-related activation, and subsequent

suppression, to air. (C) Prediction of saline or bitter had little or no effect on suppression. (D) Firing rate [150 to 300 ms; shaded region in (C)] of each neuron to predicted and unpredicted saline or bitter (with baseline rates subtracted). The diagonal indicates identity. Red triangles indicate a significant difference between responses to predicted and unpredicted saline-bitter.

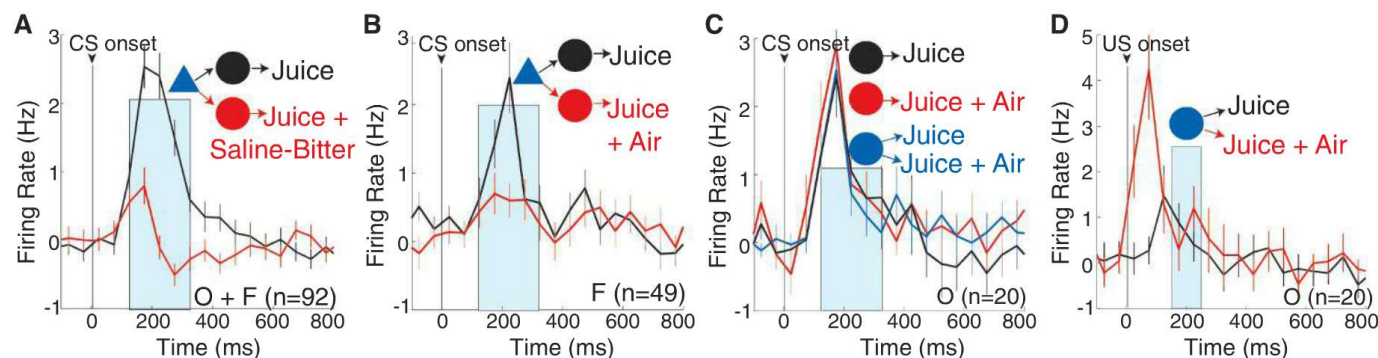


Fig. 3. In the context of juice, dopamine neurons are highly sensitive to saline and bitter, but not air. One CS predicted juice alone (180 μ l), and another predicted simultaneous delivery of juice plus an aversive stimulus (insets and fig. S1, C and D). (A) Prediction of saline (or bitter) suppressed activation to CS onset in 92 neurons from monkeys O and F. (B) Prediction of air caused only a modest suppression of activation to CS onset in monkey F. (C) Prediction of air

in monkey O had no effect. Unlike (A) and (B), no cue predicted CS onset. (D) After the "blue" CS in (C), firing rates did not discriminate delivery of air plus juice from juice alone during the period of 150 to 250 ms after unconditioned stimulus (US) onset, in which reward value is best discriminated. The short latency activation to air (40 to 100 ms) is due to its high sensory intensity and was more prominent in monkey O than in monkey F (16).

Monkeys learned that one CS predicted only juice, whereas another predicted juice plus a simultaneous aversive stimulus (fig. S1C). Consistent with both hypotheses, the CS predicting juice alone caused a much stronger activation than the CS predicting juice plus saline or bitter (Fig. 3A). However, in contrast to the single-dimension hypothesis, prediction of air caused only a small suppression of the CS response in monkey F (Fig. 3B) and no suppression in monkey O (Fig. 3C). Across all 49 cells in monkey F in which experiments were performed both with air (Fig. 3B) and saline (Fig. 3A), the effect of saline (in suppressing firing rates) was significantly greater than that of air ($P = 0.02$, paired t test). When air was delivered together with juice but with a probability of 0.5 (following a CS), its aversiveness had no effect on firing rate (Fig. 3D). Analogous experiments in which a loud (90 dB) but neutral sound replaced air yielded similar results, with the sound being ineffectual (fig. S3).

The insensitivity of dopamine neurons to aversiveness suggests that other neurons should represent aversiveness. Reward and aversiveness could be represented independently by discrete sets of neurons because they are experienced by the brain as statistically independent of one another, displaying neither strong positive nor negative correlations. They would not be represented as opposites along a single dimension because in general, they are not anticorrelated with one another. This is essentially the same explanation that has been given for receptive field formation in sensory systems, in which distinct neurons learn to recognize statistically independent features as discrete “objects” (26, 27).

Past and present results do support the existence of opponent representations for reward (3, 13, 16, 28), and the same is likely to be the case for aversiveness. Thus, one can infer from the present results that there are four types of value representations, which could be denoted as reward-ON (R_{ON}), reward-OFF (R_{OFF}), aversive-ON (A_{ON}), and aversive-OFF (A_{OFF}). The “ON” neurons would be activated by evidence for reward, or for aversiveness, and the “OFF” neurons by evidence against reward, or against aversiveness. These four putative types of neurons would mediate the four types of reinforcement distinguished at the behavioral level. Skinner denoted these, esoterically, as positive reinforcement (R_{ON}), positive punishment (A_{ON}), negative reinforcement (A_{OFF}), and negative punishment (R_{OFF}) (1). Because dopamine represents R_{ON} , it is natural to ask whether the other three major modulatory neurotransmitters might represent the other three value signals. Although some recordings have been made from neurons containing norepinephrine, serotonin, and acetylcholine, it remains uncertain how they represent value, in part because of the challenges described above in characterizing neuronal responses to both reward and aversiveness. Regardless of the other classic neuromodulators, the present results sug-

gest the existence of at least three other modulatory signals to represent the other three aspects of value and to “teach” value throughout large parts of the brain in a manner analogous to that proposed for dopamine (2, 7, 9).

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23. Of 195 dopamine neurons, 134 were from monkey O, and 61 were from monkey F. It was estimated that 81, 13, and 6% were in substantia nigra, ventral tegmental area, and retrorubral field, respectively, with 45% of all neurons being in the “ventral tier” (ventral substantia nigra compacta and reticulata) and the other 55% being in the “dorsal tier” (27).
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Supplementary Materials

www.sciencemag.org/cgi/content/full/341/6145/546/DC1
Materials and Methods
Figs. S1 to S3
Reference (29)

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Functional Lysine Modification by an Intrinsically Reactive Primary Glycolytic Metabolite

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The posttranslational modification of proteins and their regulation by metabolites represent conserved mechanisms in biology. At the confluence of these two processes, we report that the primary glycolytic intermediate 1,3-bisphosphoglycerate (1,3-BPG) reacts with select lysine residues in proteins to form 3-phosphoglyceryl-lysine (pgK). This reaction, which does not require enzyme catalysis, but rather exploits the electrophilicity of 1,3-BPG, was found by proteomic profiling to be enriched on diverse classes of proteins and prominently in or around the active sites of glycolytic enzymes. pgK modifications inhibit glycolytic enzymes and, in cells exposed to high glucose, accumulate on these enzymes to create a potential feedback mechanism that contributes to the buildup and redirection of glycolytic intermediates to alternate biosynthetic pathways.

Regulation of protein structure and function by reversible small-molecule binding (1, 2) and covalent posttranslational modification (PTM) (3) are core tenets in biochemistry. Many intermediates in primary metabolic pathways reversibly bind to proteins as a form of feedback or feedforward regulation (2). Covalent PTMs are, however, typically introduced onto pro-

teins by enzyme-catalyzed processes, but can also result from enzyme-independent interactions between reactive metabolites and nucleophilic residues in proteins (4–7). The scope and broad functional significance of nonenzymatic modifications of proteins, however, remain poorly understood. In this context, we wondered whether intrinsically reactive intermediates in primary

metabolic pathways might covalently modify proteins.

A survey of primary metabolites with the potential to modify proteins focused our attention on the central glycolytic intermediate 1,3-bisphosphoglycerate (1,3-BPG), a product of catalysis by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that has a highly electrophilic acylphosphate group (Fig. 1A). Acylphosphate reactivity is central to several enzyme-catalyzed metabolic processes (8, 9) and has proven useful in the design of electrophilic nucleotide probes that react with conserved lysines within kinase active sites (10). We thus examined whether 1,3-BPG might modify lysine residues on proteins to form 3-phosphoglyceroyl-lysine (pgK) (Fig. 1A).

Because of its propensity for rearrangement to the more stable isomer 2,3-bisphosphoglycerate (2,3-BPG), 1,3-BPG is not commercially available. Therefore, to initially determine whether 1,3-BPG reacted with proteins to form pgK modifications, we produced this metabolite *in situ* by incubating purified human GAPDH with substrate and cofactor (fig. S1). GAPDH was then trypsinized and analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) on an Orbitrap Velos mass spectrometer for peptides with a differential modification mass of 167.98238 daltons on lysines, the expected mass shift caused

by pgK formation. Several pgK-modified GAPDH peptides were identified in reactions with substrate and cofactor (GGN conditions) (Fig. 1B and table S1). These pgK-modified peptides were much less abundant, but still detectable in control reactions lacking substrate (GN) or cofactor (GG), which suggested that commercial GAPDH, which is purified from erythrocytes, may be constitutively pgK-modified. Structural assignments for two distinct pgK-modified GAPDH peptides were verified by comparison with synthetic peptide standards (fig. S2, see Materials and Methods), which showed equivalent LC retention times and MS/MS spectra (Fig. 1C and figs. S3 and S4). Analysis of a GAPDH crystal structure revealed that all of the pgK-modified lysines are solvent-exposed (fig. S5) and that the most frequently identified sites of modification (K107, K194, and K215) (table S1) cluster around the GAPDH active site (Fig. 1D). Isoelectric focusing (IEF) revealed a shift in the isoelectric point (pI) distribution of GAPDH from ~8.6 in GN control reactions to 6.5 to 7.66 in GGN reactions (Fig. 1E and fig. S6). This shift is consistent with GAPDH's having acquired a net negative charge through capping of lysines by phosphoglycerate, a conclusion also supported by LC-MS/MS analysis, which revealed substantial enrichment of pgK-modified peptides in the acidic IEF fractions (fig. S6).

We next assessed the existence and global distribution of pgK modifications in cell proteomes. We reasoned that pgK-peptides might share enough physicochemical properties with phosphorylated peptides to permit enrichment

by a standard phosphoproteomic workflow that used immobilized metal affinity chromatography (IMAC) (fig. S7) (11). pgK-modified lysines were identified in several protein classes in four human cell lines examined (table S2). Two of the aforementioned pgK-sites observed for GAPDH *in vitro* were detected in human cells and generated MS/MS spectra that matched the spectra of both the synthetic (fig. S8) and *in vitro*-derived (figs. S3 and S4) pgK-modified GAPDH peptides. Meta-analysis of published phosphoproteomic data sets (12, 13) confirmed that pgK-modified proteins are also present in normal mouse tissues and that several pgK sites are conserved in human and mouse protein orthologs (Fig. 2, A and B, and table S3). Bioinformatic clustering with DAVID software (14) and KEGG pathway analysis revealed enrichment of pgK-modified proteins in glycolysis in both human cells (Fig. 2C and table S4) and mouse liver (Fig. 2D and table S4).

Examination of the observed modification sites revealed that they often occurred on catalytic or regulatory lysine residues in the active sites of glycolytic enzymes that use three-carbon substrates (Fig. 2, A, B, and E; and fig. S9). Notable exceptions were phosphoglycerate kinase (PGK1) and bisphosphoglycerate mutase (BPGM), both of which accept 1,3-BPG as a substrate but have active sites predominantly made up of histidine and arginine residues (fig. S10). These findings suggest that both PGK1 and BPGM may have evolved to have active sites that are resistant to stable modification by 1,3-BPG. Analysis of the local sequence context surrounding

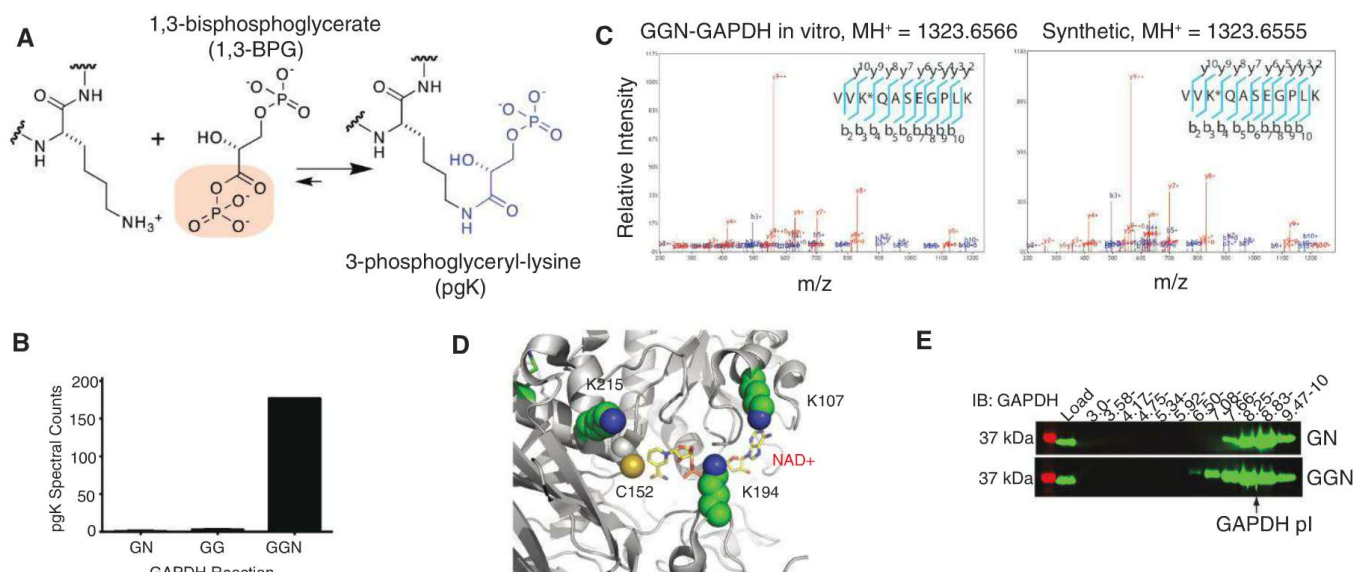


Fig. 1. 1,3-BPG forms a stable, covalent modification on lysines of GAPDH *in vitro*. (A) pgK formed by reaction of a lysine ε-amine with the acylphosphate functionality in 1,3-BPG. (B) Spectral counts of pgK-modified tryptic peptides detected by LC-MS/MS analyses of GN, GG, and GGN GAPDH enzymatic reactions (average of two independent experiments). (C) MS/MS spectra of the *in vitro* GGN-GAPDH-derived (left) and synthetic (right) doubly charged tryptic peptide VV(pg)KQASEGPLK. Observed b-, y-, and relevant

parent ions, as well as products of dehydration (°) or ammonia loss (*) are labeled. Asterisk (*) within peptide sequences denotes the pgK-modified lysine. (D) The most frequently detected pgK-modification sites (K107, K194, and K215) surround the active site of GAPDH [Protein Data Bank (PDB) accession no. 1ZNIQ]. (E) Western immunoblot (IB) with antibody against GAPDH in GN- and GGN-GAPDH reactions after IEF analysis. Data are from a representative experiment of three independent experiments.

pgK sites revealed no discernible motif (fig. S11), which is consistent with an enzyme-independent labeling mechanism.

Metabolic labeling with heavy glucose (D -glucose- $^{13}C_6$, 1, 2, 3, 4, 5, 6, 6- d_7) revealed that pgK modifications were derived from glucose metabolism (fig. S12). We next tested whether changes in glycolytic flux, through altering 1,3-BPG, might dynamically regulate the extent of pgK modifications in cells. We exposed cells to normal (10 mM) or high (25 mM) concentrations of glucose and found that the latter cells, which had 4- to 5-fold elevations in bis-(1,3 and/or 2,3)-phosphoglycerate (Fig. 3A), exhibited greater pgK signals for several proteins (Fig. 3B), as judged by immunoblotting with antibodies generated against pgK (fig. S13 and Materials and Methods). Similar experiments performed on human embryonic kidney-293T (HEK293T) cells expressing FLAG-tagged ENO1 or GAPDH revealed that both proteins showed significant glucose concentration-dependent increases in their pgK-modification state (Fig. 3, C and D). We also used the quantitative proteomic method SILAC [stable isotope labeling of amino acids in cell culture (15)] and found that cells grown in 25 mM glucose exhibited increased pgK modification of several proteins, including the active-site lysine of ENO1, K343, without changes in the abundance of non-pgK peptides in these

proteins (Fig. 3, E and F; table S5; and fig. S14). IEF further established a shift in ENO1 protein to more acidic pH fractions in cells grown in 25 mM glucose (Fig. 3G). Finally, analysis of mouse phosphoproteomic data sets (12) revealed that the extent and distribution of pgK modifications for glycolytic enzymes, as measured by pgK-peptide spectral counts, were highest in liver, brain, and kidney, which are major sites of glucose uptake and glycolytic and gluconeogenic activity in vivo (Fig. 3H, fig. S15, and table S3) (16).

We detected pgK modification of multiple nuclear proteins (tables S2 and S3 and figs. S14C and S16A). GAPDH can localize to the nucleus (17), and this distribution is promoted by exposing cells to high concentrations of glucose (18, 19) (fig. S16, B and C), which provides a potential mechanistic explanation for the glucose-stimulated increase in pgK signals for the nuclear protein NUCKS1 (nuclear ubiquitous casein and cyclin-dependent kinases substrate 1) (figs. S14C and S16D). We further tested this premise by comparing nuclear pgK profiles in HEK293T cells transfected with wild-type (GAPDH-WT) or nuclear-localized GAPDH (GAPDH-NLS, containing a C-terminal SV40 nuclear localization sequence) (fig. S16, E to G). GAPDH-NLS cells exhibited increased pgK signals in a subset of nuclear proteins (fig. S16H), including NUCKS1 (fig. S16I).

We next assessed the impact of pgK modification on glycolytic enzyme activity. We first compared in vitro pgK-modified (GGN) with control (GN) GAPDH reactions after dialysis to remove unreacted 1,3-BPG and other metabolites. GAPDH from GGN reactions exhibited an approximately twofold increase in apparent Michaelis constant (K_m) value (Fig. 4A), indicating that pgK modifications may perturb interactions with GAP substrate. We attempted to mimic 1,3-BPG modification of the catalytic lysine (K343) of ENO1 (20), as well as another active site lysine (K394) (Fig. 4B), by generating K-to-E mutations, which displayed substantially reduced activity (Fig. 4C and fig. S17A). We also found that FLAG-tagged ENO1 enzyme expressed in HEK293T cells showed ~30% lower activity after exposure to 25 mM versus 10 mM glucose for 24 hours (Fig. 4D), which is consistent with our quantitative proteomic findings indicating enhanced pgK modification of K343 in cells exposed to high glucose concentrations (Fig. 3C, E-G).

We used targeted metabolomics (table S6) to measure glycolytic and citric acid cycle (CAC) intermediates in human cells exposed to 10 versus 25 mM glucose for 24 hours and, as expected, found higher concentrations of metabolites in the latter condition. However, these increases were not uniform but were mostly restricted to central

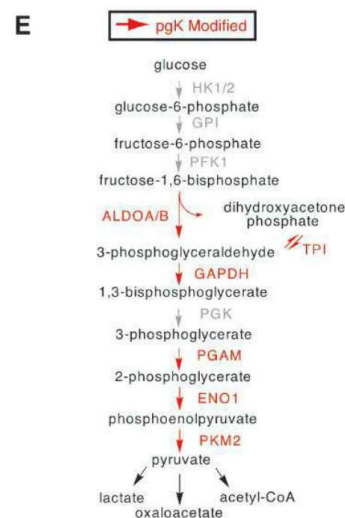
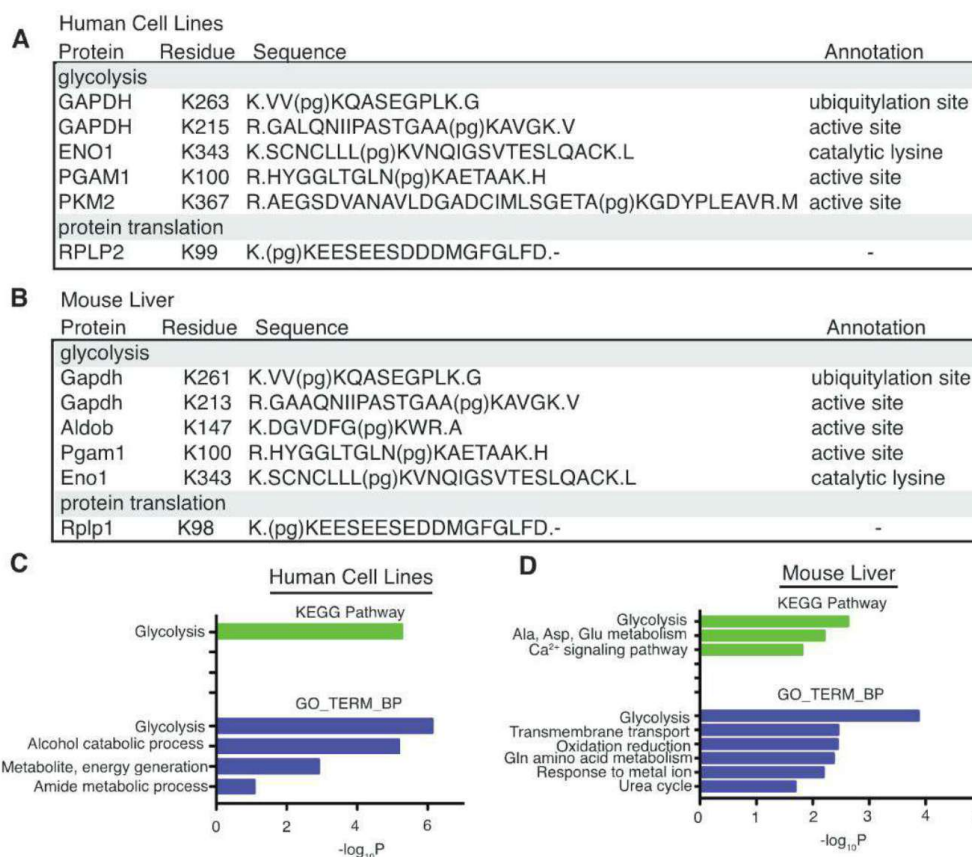


Fig. 2. Functional distribution of pgK-modification sites in human cells and mouse tissues. (A and B) Modification site, peptide sequence, and associated annotation for representative endogenous pgK-modified proteins from human cell lines (A) and mouse liver (B). "(pg)K" denotes the pg-modified lysine. **(C and D)** Gene ontology biological process categories (GOTERM_BP) and KEGG pathways enriched among pgK-modified proteins in human cell lines (C) and mouse liver

(D) by DAVID bioinformatic analysis. **(E)** Schematic of observed pgK-modified enzymes in glycolysis. Glycolytic enzymes containing at least one pgK site are shown in red, others are shown in gray.

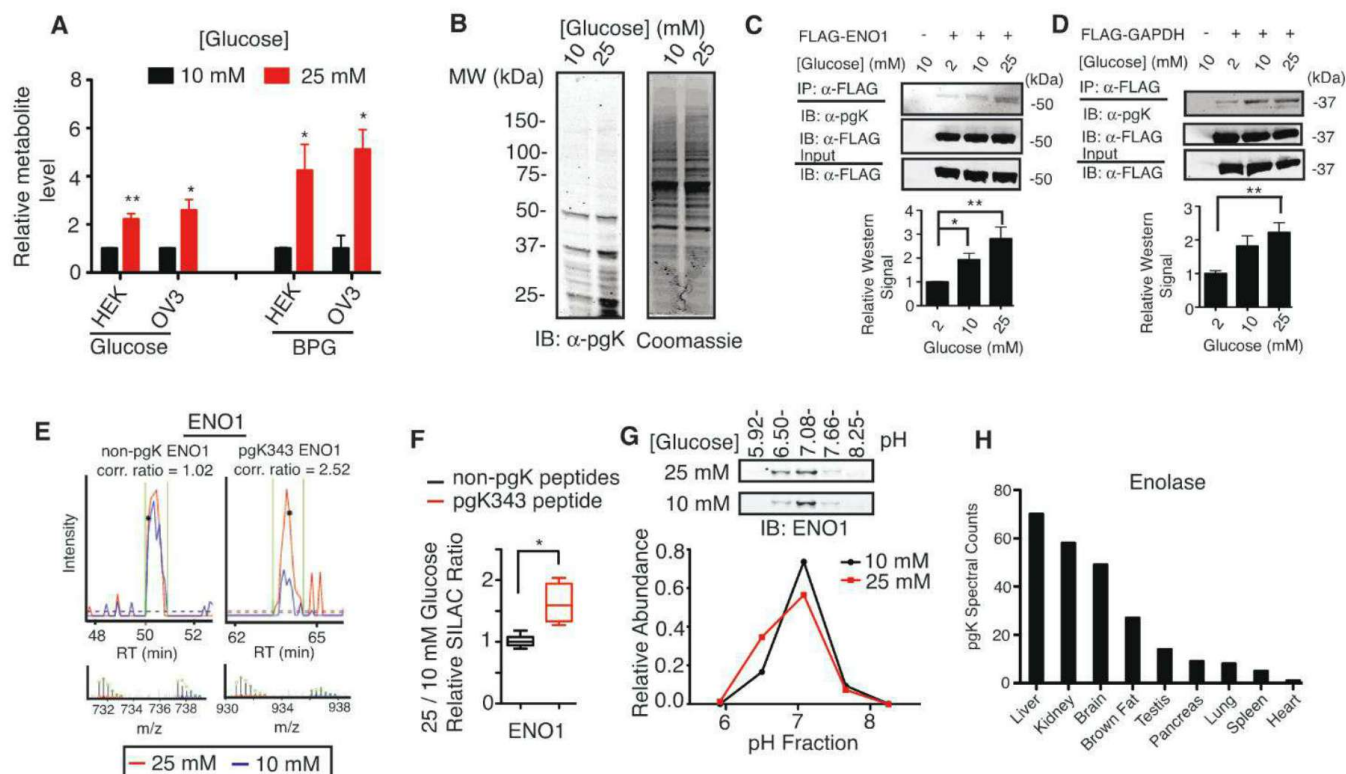


Fig. 3. Dynamic coupling of pgK modification to glucose metabolism.

(A) Intracellular glucose and bisphosphoglycerate (BPG, aggregate of both 1,3- and 2,3-isomers) levels from cells grown at indicated glucose concentrations for 24 hours. (B) Antibody against pgK (α -pgK) IB and Coomassie-stained gel of proteomes from HEK293T cells grown at indicated glucose concentrations. (C and D) α -pgK IB of α -FLAG-enriched ENO1 (C) and GAPDH (D) expressed in HEK293T cells grown at indicated glucose concentrations. Shown below each blot is a graph of the average relative α -pgK band intensities ($n = 4$ per group). (E) Representative SILAC chromatograms, MS1 isotope envelopes, and corrected (corr.) area ratios for non-pgK (left) and pgK-modified (right) peptides from ENO1. Integration area is shown within

green bars; asterisk (*) in the chromatogram signifies the triggered MS2 scan. (F) Average SILAC ratios for pgK343-containing and non-pgK ENO1 peptides from cells grown at indicated glucose concentrations. Horizontal line and whiskers represent the mean and 10 to 90% confidence intervals, respectively. (G) IB of IEF-focused ENO1 from MCF7 cells grown at the indicated glucose concentrations. Plot shows the IEF-focused ENO1 pI distributions quantified by densitometry. (H) Spectral count values for pgK-modified enolase peptides across nine mouse tissues (table S3). Data represent means $\pm$ SEM; statistical significance was determined by two-way t tests with a Bonferroni correction for (C) and (D) and Welch's correction for (F): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

glycolytic metabolites, including fructose-1,6-bisphosphate (FBP), glyceraldehyde-3-phosphate (GAP), phosphoglycerate (both 2- and 3-isomers, 2PG and 3PG) and phosphoenolpyruvate (PEP) (Fig. 4E and fig. S17, B to D). A similar metabolomic profile was observed in cells cultured in normal glucose and treated with the ENO1 and PKM2 inhibitor NaF (3 mM, 24 hours) (fig. S17, E to G). We used pulse-chase experiments with 10 mM ^{13}C -labeled glucose to measure glycolytic flux in cells grown in 10 versus 25 mM glucose for 24 hours. Cells grown in 25 mM glucose showed reduced glycolytic flux at early time points postchase with ^{13}C -glucose (≤ 6 hours) and these decreases were alleviated by 16 hours (Fig. 4F), which correlated with changes in pgK-signals over the time course (fig. S17H, I). Finally, the build up of glycolytic metabolites in cells exposed to either high glucose or NaF was accompanied by increased concentrations of metabolites that are biosynthesized from central glycolytic intermediates, including serine, ribulose-5-phosphate, and reduced glutathione (Fig. 4G and fig. S17J).

We found that a synthetic, pgK-containing tetrapeptide Ac-AA(pg)KA (fig. S18A) was deacylated (Ac-AAKA) and dephosphorylated [Ac-AA(g)KA; glycyl-lysine, (g)K] when exposed to native, but not heat-denatured, cell lysates or buffer alone (fig. S18, B and C), which indicated that pgK modifications can be enzymatically metabolized. Broad-spectrum phosphatase inhibitors blocked formation of dephosphorylated and, to a lesser extent, deacylated peptide products (fig. S18D), which suggested that deacylation may occur directly from pgK-peptides or through a dephosphorylated gK intermediate (fig. S18E). Kinetic analysis in cell lysates further revealed that deacylation and dephosphorylation reactions occur on similar time scales in cell lysates (fig. S18F). These data indicate that pgK is a dynamic and reversible PTM.

In summary, we have identified herein a PTM found on numerous mammalian proteins (tables S2 and S3) that originates from the reaction of lysines with a primary glycolytic metabolite, 1,3-BPG. This reaction increases the size and inverts the charge potential of the modified residue from

positive to negative and, therefore, has the potential to affect the structure and function of proteins from diverse families and pathways (fig. S19). 1,3-BPG modification of lysines also likely occurs in lower organisms, given the conservation of glycolysis throughout evolution (21, 22). The enrichment of pgK-modification sites on glycolytic enzymes indicates that these enzymes may form a physical complex in cells (23), as has been observed for other metabolic pathways (24). The localization of GAPDH to additional subcellular structures or protein complexes could provide a general mechanism to regulate the pgK-modification state of proteins. An aggregate effect of partial enzyme impairments by pgK modification in the glycolytic pathway may be reduced carbon flow into Lac and CAC intermediates, which leads to increased levels of central metabolites that can be shunted to alternate biosynthetic pathways (fig. S20) (25, 26). Erythrocytes isolated from patients with a rare deficiency in BPGM, which should increase 1,3-BPG concentrations, show a profile similar to that of cells grown in high concentrations of glucose, with build-up of

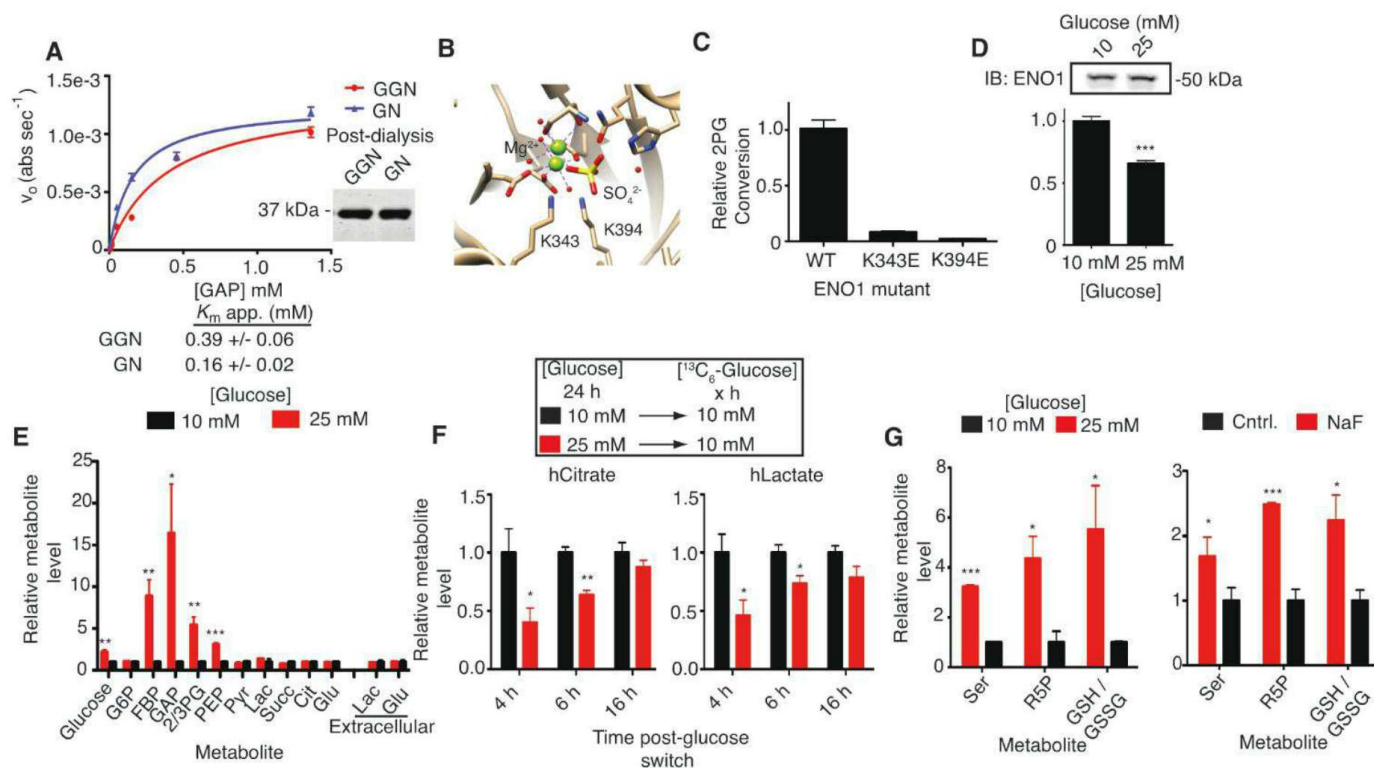


Fig. 4. pgK modification impairs glycolytic enzymes and correlates with altered glycolytic output in human cells. (A) Michaelis-Menten kinetic analysis comparing GAPDH from GGN (1,3-BPG-producing) with GN (control) reactions. v_0 , initial enzyme velocity measurements. (B) Structure of ENO1 active site (PDB accession no. 3B97) showing residues important for catalysis, including pgK sites K343 and K394. (C) Relative activities of wild-type and mutant FLAG-tagged ENO1 expressed and affinity-isolated from HEK293T cells (fig. S17A for expression data of ENO1 variants). (D) Relative activities of FLAG-isolated ENO1 expressed in HEK293T cells cultured in 10 versus 25 mM glucose for 24 hours. (E) Relative metabolite levels in HEK293T cells

grown in 10 versus 25 mM glucose for 24 hours. (F) Relative heavy lactate and citrate levels in HEK293T cells pretreated with the indicated glucose concentrations for 24 hours and then grown in 10 mM heavy glucose for the indicated length of time. (G) Relative metabolite measurements in cells grown in 10 versus 25 mM glucose (left) or treated with NaF (right). Data shown represent means $\pm$ SEM from triplicate experiments. Statistical significance was determined by two-way t tests: * P < 0.05; ** P < 0.01; *** P < 0.005; n.s., not significant. G6P, glucose-6-phosphate; FBP, fructose-1,6-bisphosphate; Pyr, pyruvate; Lac, lactate; Succ, succinate; Cit, citrate; Glu, glutamate; Ser, serine; R5P, ribulose-5-phosphate; GSH/GSSG, reduced/oxidized glutathione.

the central metabolites FBP, glyceraldehyde-3-phosphate (GAP), 2PG, 3PG, and PEP (27). Thus, pgK modifications could constitute an intrinsic feedback mechanism by which a reactive central metabolite (1,3-BPG) regulates product distribution across the glycolytic pathway in response to changes in glucose uptake and metabolism.

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- A review of literature on glycolytic enzyme modification revealed evidence for phosphate-modified forms of GAPDH and ENO1 in bacteria, which were interpreted to potentially correspond to enzymes modified by adenosine diphosphate ribosylation and substrate (2-phosphoglycerate), respectively (22). Our data offer an additional interpretation of these findings as evidence that 1,3-BPG may also modify glycolytic enzymes in bacteria.
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Supplementary Materials

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Cytochrome P450 Drives a HIF-Regulated Behavioral Response to Reoxygenation by *C. elegans*

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Oxygen deprivation followed by reoxygenation causes pathological responses in many disorders, including ischemic stroke, heart attacks, and reperfusion injury. Key aspects of ischemia-reperfusion can be modeled by a *Caenorhabditis elegans* behavior, the O₂-ON response, which is suppressed by hypoxic preconditioning or inactivation of the O₂-sensing HIF (hypoxia-inducible factor) hydroxylase EGL-9. From a genetic screen, we found that the cytochrome P450 oxygenase CYP-13A12 acts in response to the EGL-9–HIF-1 pathway to facilitate the O₂-ON response. CYP-13A12 promotes oxidation of polyunsaturated fatty acids into eicosanoids, signaling molecules that can strongly affect inflammatory pain and ischemia-reperfusion injury responses in mammals. We propose that roles of the EGL-9–HIF-1 pathway and cytochrome P450 in controlling responses to reoxygenation after anoxia are evolutionarily conserved.

Ischemia-reperfusion-related disorders, such as strokes and heart attacks, are the most common causes of adult deaths worldwide (1). Blood delivers O₂ and nutrients to target tissues, and ischemia results when the blood supply is interrupted. The restoration of O₂ from blood flow after ischemia, known as reperfusion, can exacerbate tissue damage (2). How organisms prevent ischemia-reperfusion injury is poorly understood. Studies of the nematode *C. elegans* led to the discovery of an evolutionarily conserved family of O₂-dependent enzymes (EGL-9 in *C. elegans* and EGLN2 in mammals) that hydroxylate the HIF transcription factor and link hypoxia to hypoxia-inducible factor (HIF)-mediated physiological responses (3–7). Exposure to chronic low concentrations of O₂ (hypoxic preconditioning) or direct inhibition of EGLN2 strongly protects mammals from stroke and ischemia-reperfusion injury (2, 8, 9). Similarly, EGL-9 inactivation in *C. elegans* blocks a behavioral response to reoxygenation, the O₂-ON response (characterized by a rapidly increased locomotion speed triggered by reoxygenation after anoxia) (10, 11), which is similar to mammalian tissue responses to ischemia-reperfusion: (i) Reoxygenation drives the O₂-ON response and is the major pathological driver of reperfusion injury, (ii) hypoxic preconditioning can suppress both processes, and (iii) the central regulators (EGL-9–HIF) of both processes are evolutionarily conserved. It remains unknown how the EGL-9–HIF-1 and EGLN2–HIF pathways control the O₂-ON response and ischemia-reperfusion injury, respectively.

To seek EGL-9–HIF-1 effectors important in the O₂-ON response, we performed an *egl-9* suppressor screen for mutations that can restore the defective O₂-ON response in *egl-9* mutants (fig. S1A). We identified new alleles of *hif-1* in this screen; because EGL-9 inhibits HIF-1, *hif-1* mutations suppress the effects of *egl-9* mutations (10). We also identified mutations that are not alleles of *hif-1* (Fig. 1, A to C, and fig. S1B). *hif-1* mutations recessively suppressed three defects of *egl-9* mutants: the defective O₂-ON response, defects in egg laying, and the ectopic expression of the HIF-1 target gene *cysl-2* (previously called *K10H10.2*) (fig. S1C) (10, 12). By contrast, one mutation, *n5590*, dominantly suppressed the O₂-ON defect but did not suppress the egg-laying defect or the ectopic expression of *cysl-2::GFP* (Fig. 1, D and E, and fig. S2). *n5590* restored the sustained phase (starting 30 s after reoxygenation) better than it did the initial phase (within 30 s after reoxygenation) (Fig. 1, A to C). *egl-9; hif-1; n5590* triple mutants displayed a normal O₂-ON response, just like the wild type and *egl-9; hif-1* double mutants (fig. S1D). Thus, *n5590* specifically suppresses the *egl-9* defect in the sustained phase of the O₂-ON response.

We genetically mapped *n5590* and identified a Met⁴⁶ → Ile missense mutation in the gene *cyp-13A12* by whole-genome sequencing (Fig. 2A, fig. S3A, and table S1A). Decreased wild-type *cyp-13A12* gene dosage in animals heterozygous for a wild-type allele and the splice acceptor null mutation *gk733685*, which truncates the majority of the protein, did not recapitulate the dominant effect of *n5590* (Fig. 2B). Similarly, *gk733685* homozygous mutants did not recapitulate the effect of *n5590* (Fig. 2C). Thus, *n5590* does not cause a loss of gene function. By contrast, increasing wild-type *cyp-13A12* gene dosage by overexpression restored the sustained phase of the O₂-ON response (Fig. 2D), and

RNA interference (RNAi) against *cyp-13A12* abolished the effect of *n5590* (Fig. 2E). We conclude that *n5590* is a gain-of-function allele of *cyp-13A12*.

cyp-13A12 encodes a cytochrome P450 oxygenase (CYP). CYPs can oxidize diverse substrates (13–15). The *C. elegans* genome contains about 82 CYP genes, at least two of which are polyunsaturated fatty acid (PUFA) oxygenases that generate eicosanoid signaling molecules (fig. S3B) (16, 17). On the basis of BLASTP scores, the closest human homolog of CYP-13A12 is CYP3A4 (fig. S4). We aligned the protein sequences of CYP-13A12 and CYP3A4 and found that *n5590* converts methionine 46 to an isoleucine, the residue in the corresponding position of normal human CYP3A4 (fig. S4). Methionines can be oxidized by free radicals, which are produced in the CYP enzymatic cycle, rendering CYPs prone to degradation (18, 19). Using transcriptional and translational green fluorescent protein (GFP)-based reporters, we identified the pharyngeal marginal cells as the major site of expression of *cyp-13A12* (fig. S5) and observed that the abundance of CYP-13A12::GFP protein was decreased by prolonged hypoxic preconditioning and also decreased in *egl-9* but not in *egl-9; hif-1* mutants (Fig. 2F and fig. S5). The *n5590* mutation prevented the decrease in CYP-13A12::GFP abundance by hypoxia or *egl-9*. Thus, *n5590* acts, at least in part, by restoring the normal abundance of CYP-13A12, which then promotes the O₂-ON response in *egl-9* mutants.

We tested whether CYP-13A12 was normally required for the O₂-ON response in wild-type animals. The *cyp-13A12* null allele *gk733685* abolished the sustained phase of the O₂-ON response; the initial phase of the O₂-ON response was unaffected (Fig. 3A). A wild-type *cyp-13A12* transgene fully rescued this defect (Fig. 3B). A primary role of CYP-13A12 in the sustained phase of the O₂-ON response explains the incomplete rescue of the defective O₂-ON response of *egl-9* mutants by *n5590* during the initial phase (Fig. 1C). The activity of most and possibly all *C. elegans* CYPs requires EMB-8, a CYP reductase that transfers electrons to CYPs (20). No non-CYP EMB-8 targets are known. The mutation *emb-8(hc69)* causes a temperature-sensitive embryonic lethal phenotype. We grew *emb-8(hc69)* mutants at the permissive temperature to the young-adult stage. A shift to the nonpermissive temperature simultaneously with *Escherichia coli*-feeding RNAi against *emb-8* nearly abolished the O₂-ON response (Fig. 3, C and D). [Both the *hc69* mutation and RNAi against *emb-8* were required to substantially reduce the level of EMB-8 (17).] CYP-13A12 is thus required for the sustained phase of the O₂-ON response, and one or more other CYPs likely act with CYP-13A12 to control both phases of the O₂-ON response.

CYP oxygenases define one of three enzyme families that can convert PUFAs to eicosanoids, which are signaling molecules that affect inflam-

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matory pain and ischemia-reperfusion responses of mammals (15, 21–23); the other two families, cyclooxygenases and lipoxygenases, do not appear to be present in *C. elegans* (17, 24). To test whether eicosanoids are regulated by EGL-9 and CYP-13A12, we used high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) to profile steady-state amounts of 21 endogenous eicosanoid species from cell extracts of wild-type, *egl-9(n586)*, and *egl-9(n586); cyp-13A12(n5590)* strains. Only free eicosanoids have potential signaling roles (21, 22, 24), so we focused on free eicosanoids. The *egl-9* mutation caused a marked decrease in the overall amount of free eicosanoids, whereas the total amount of eicosanoids, including both free and membrane-bound fractions, was unaltered (Fig. 4A and fig. S6). Among the eicosanoids profiled, 17,18-DiHEQ (17,18-diolhydroxyeicosatetraenoic acid) was the most abundant species (fig. S6B). 17,18-DiHEQ is the catabolic hydrolase product of 17,18-EEQ (17,18-epoxyeicosatetraenoic acid), an epoxide active in eicosanoid signaling (25). Free cytosolic

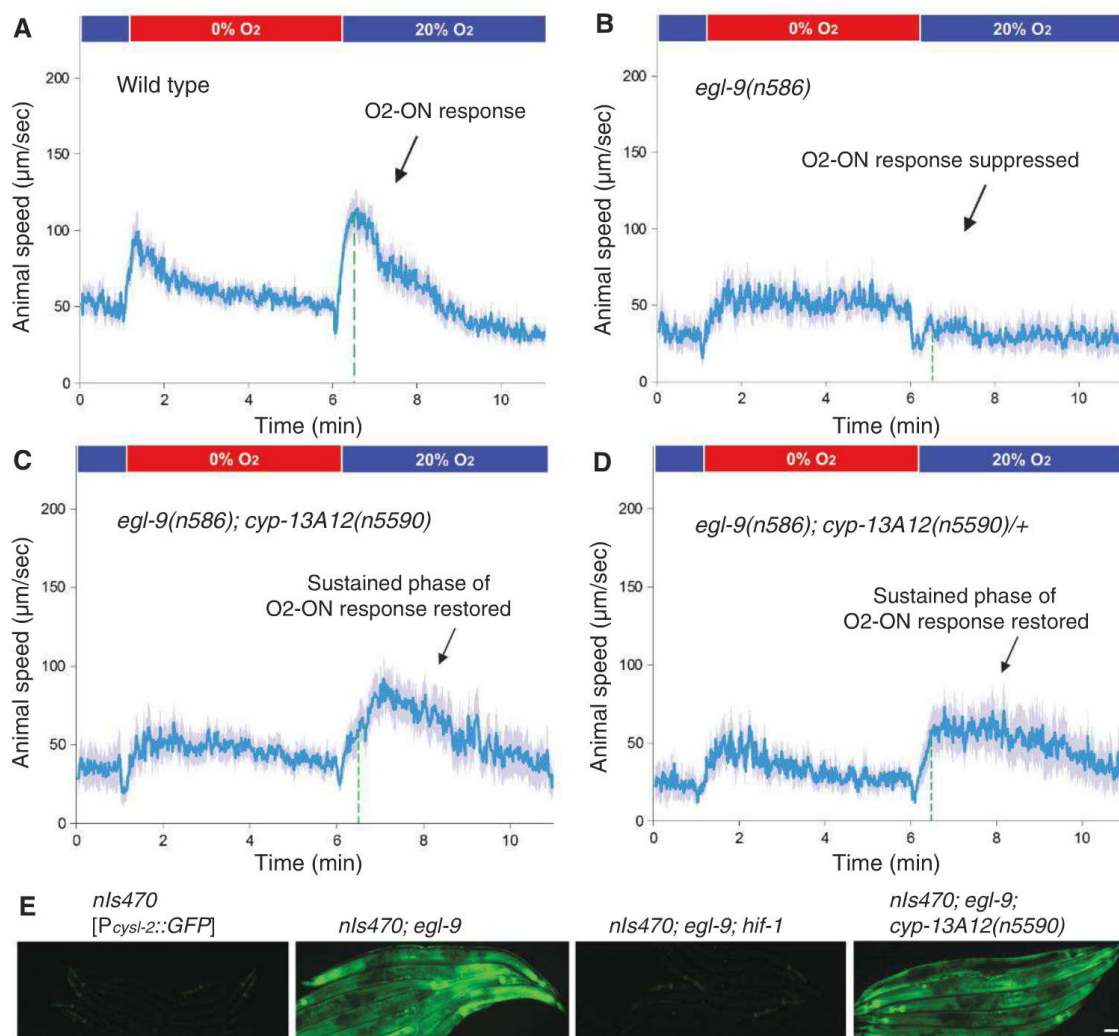
17,18-EEQ and 19-hydroxyeicosatetraenoic acid (19-HETE) were present in the wild type but undetectable in *egl-9* mutants (Fig. 4, C to F). *egl-9(n586); cyp-13A12(n5590)* mutants exhibited partially restored free overall eicosanoid levels as well as restored levels of 17,18-EEQ and 19-HETE (Fig. 4, A to F, and fig. S6B). Thus, both EGL-9 and CYP-13A12 regulate amounts of free cytosolic eicosanoids.

We tested whether the O₂-ON response requires PUFAs, which are CYP substrates and eicosanoid precursors. PUFA-deficient *fat-2* and *fat-3* mutants (26) exhibited a complete lack of the O₂-ON response, although the acceleration in response to anoxia preceding the O₂-ON response was normal (Fig. 4G and fig. S7, A to C). The defective O₂-ON response of *fat-2* mutants was restored by feeding animals arachidonic acid, a C20 PUFA (Fig. 4H), but not oleate, a C18 monounsaturated fatty acid that is processed by FAT-2 to generate C20 PUFAs (fig. S7D). These results demonstrate an essential role of PUFAs for the O₂-ON response.

We suggest a model in which CYPs, which are strictly O₂-dependent (27, 28), generate eicosanoids to drive the O₂-ON response (Fig. 4I and fig. S8). In this model, EGL-9 acts as a chronic O₂ sensor, so that during hypoxic preconditioning, the O₂-dependent activity of EGL-9 is inhibited, HIF-1 is activated, and unknown HIF-1 up-regulated targets decrease CYP protein abundance. The low abundance of CYPs defines the hypoxic preconditioned state. Without hypoxic preconditioning, CYPs generate eicosanoids, which drive the O₂-ON response. By contrast, with hypoxic preconditioning or in *egl-9* mutants, the CYP amounts are insufficient to generate eicosanoids and the O₂-ON response is not triggered. Neither C20 PUFAs nor overexpression of CYP-29A3 restored the defective O₂-ON response of *egl-9* mutants (figs. S9 and S10), indicating that this defect is unlikely to be caused by a general deficiency in C20 PUFAs or CYPs. Because the O₂-ON response requires EMB-8, a general CYP reductase, but only the sustained phase requires CYP-13A12, we propose

Fig. 1. *n5590* suppresses the defect of *egl-9* mutants in the O₂-ON response.

(A) Speed graph of wild-type animals, showing a normal O₂-ON response. Average speed values $\pm$ 2 SEM (blue) of animals ($n > 50$) are shown with step changes of O₂ between 20 and 0% at the indicated times. The mean speed within 0 to 120 s after O₂ restoration is increased relative to that before O₂ restoration ($P < 0.01$, one-sided unpaired *t* test). The dashed green line indicates the approximate boundary (30 s after reoxygenation) between the initial and sustained phases of the O₂-ON response. (B) Speed graph of *egl-9(n586)* mutants, showing a defective O₂-ON response. (C) Speed graph of *egl-9(n586); cyp-13A12(n5590)* mutants, showing a restored O₂-ON response mainly in the sustained phase (right of the dashed green line). The mean speed within 30 to 120 s after O₂ restoration was significantly higher than that of *egl-9(n586)* mutants ($P < 0.01$). (D) Speed graph of *egl-9(n586); cyp-13A12(n5590)/+* mutants, showing a restored O₂-ON response in the sustained phase. (E) *hif-1* but not *cyp-13A12(n5590)* suppressed the expression of *cysl-2::GFP* by *egl-9(n586)* mutants. GFP fluorescence micrographs of five to seven worms aligned side by side carrying the transgene *nls470* [*P_{cysl-2}::GFP*] are shown. Scale bar, 50 μ m.



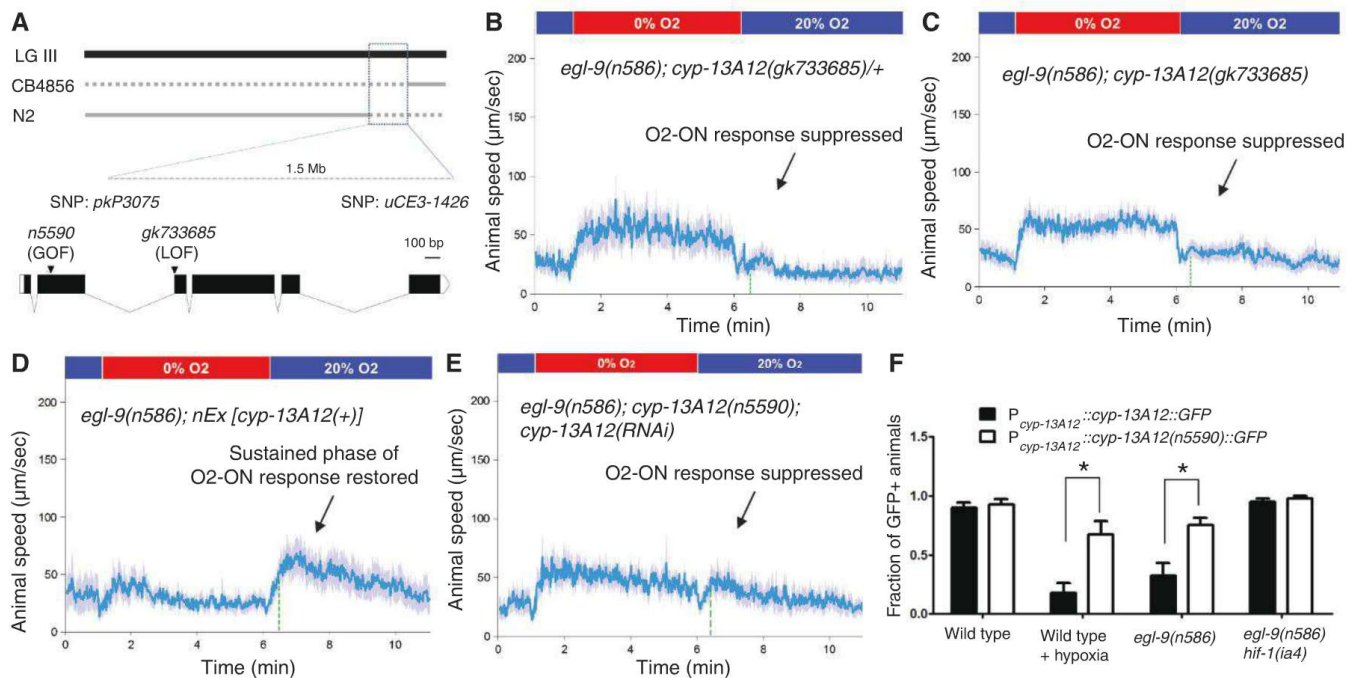
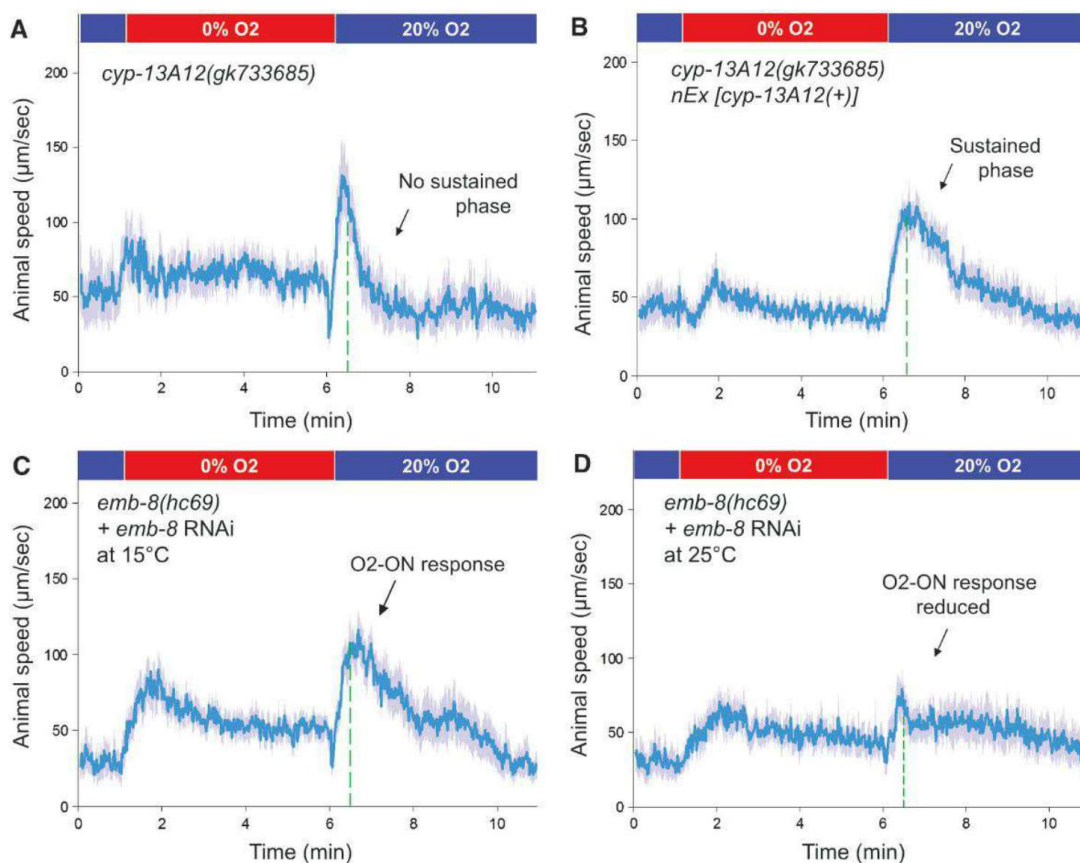


Fig. 3. Requirement of CYP-13A12 for a normal O₂-ON response.

(A) Speed graph of *cyp-13A12(gk733685)* loss-of-function mutants, showing an O₂-ON response with a normal initial phase but a diminished sustained phase (left and right, respectively, of the dashed green line). (B) Speed graph of *cyp-13A12(gk733685)* mutants with a rescuing wild-type *cyp-13A12* transgene, showing the O₂-ON response with a normal initial phase and sustained phase. The mean speed within 30 to 120 s after O₂ restoration was higher than that of *cyp-13A12(gk733685)* mutants (*P* < 0.01, one-sided unpaired *t* test, *n* > 50). (C) Speed graph of *emb-8(hc69)* mutants grown at the permissive temperature of 15°C with simultaneous *E. coli*-feeding RNAi against *emb-8*, showing a normal O₂-ON response. (D) Speed graph of *emb-8(hc69)* mutants grown post-embryonically at the restrictive temperature of 25°C with simultaneous *E. coli*-feeding RNAi against *emb-8*, showing a reduced O₂-ON response.



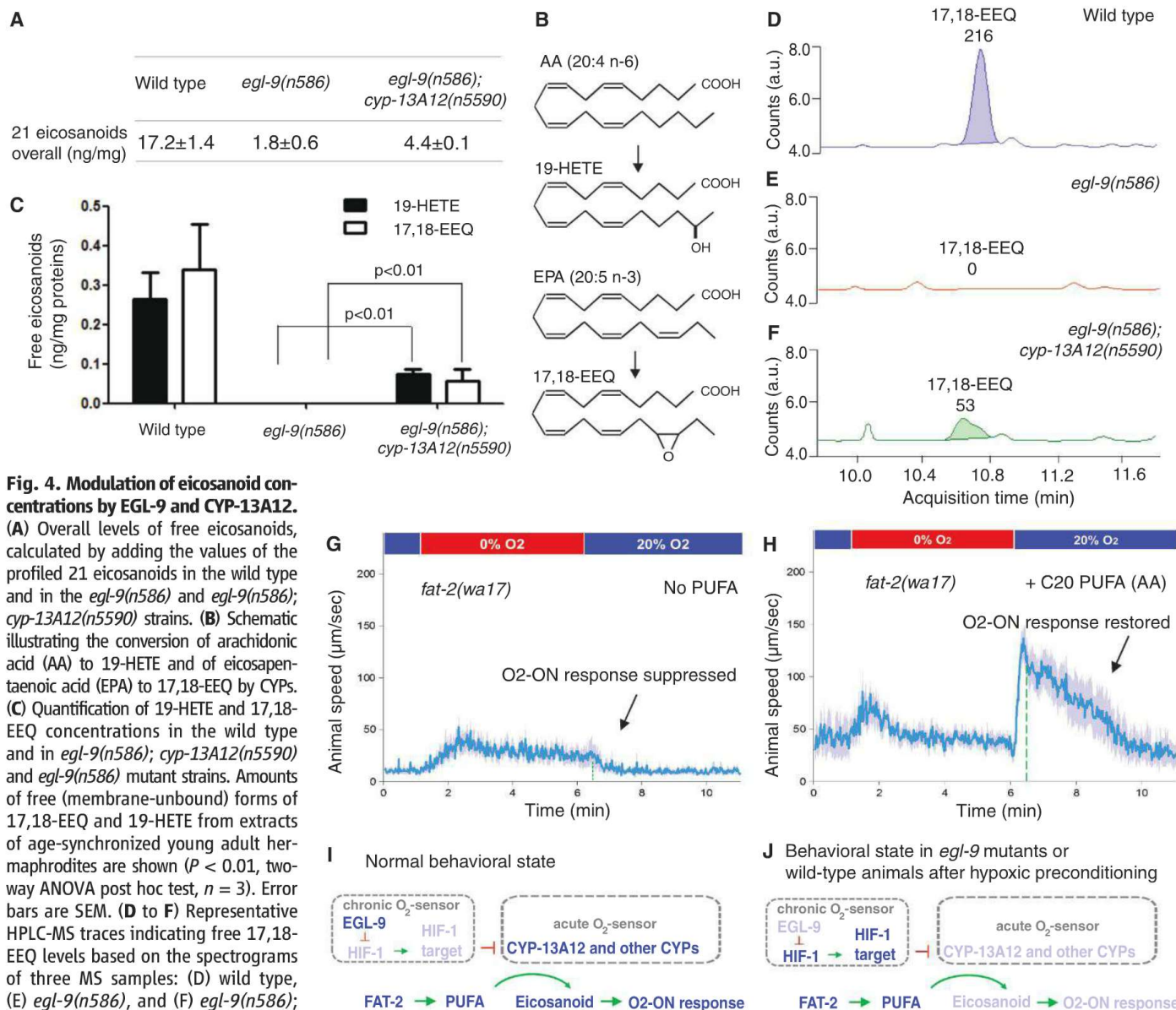


Fig. 4. Modulation of eicosanoid concentrations by EGL-9 and CYP-13A12.

(A) Overall levels of free eicosanoids, calculated by adding the values of the profiled 21 eicosanoids in the wild type and in the *egl-9(n586)* and *egl-9(n586); cyp-13A12(n5590)* strains. (B) Schematic illustrating the conversion of arachidonic acid (AA) to 19-HETE and of eicosapentaenoic acid (EPA) to 17,18-EEQ by CYPs. (C) Quantification of 19-HETE and 17,18-EEQ concentrations in the wild type and in *egl-9(n586); cyp-13A12(n5590)* and *egl-9(n586)* mutant strains. Amounts of free (membrane-unbound) forms of 17,18-EEQ and 19-HETE from extracts of age-synchronized young adult hermaphrodites are shown ($P < 0.01$, two-way ANOVA post hoc test, $n = 3$). Error bars are SEM. (D to F) Representative HPLC-MS traces indicating free 17,18-EEQ levels based on the spectrograms of three MS samples: (D) wild type, (E) *egl-9(n586)*, and (F) *egl-9(n586); cyp-13A12(n5590)*. Peaks of 17,18-EEQ at its transition m/z (mass-to-charge ratio) were measured and extracted (MassHunter). The x axis shows the retention time (minutes); the y axis shows the abundance (counts), with specific integral values over individual peaks indicated above each peak. (G) Speed graph of *fat-2* mutants, showing a defective O₂-ON response. Animals were supplemented with the solvents used in (H) as a control. (H) Speed graph of *fat-2* mu-

nants, showing the O₂-ON response rescued by C20 PUFA (AA) supplementation. (I and J) Model of how EGL-9 and CYPs control the O₂-ON response under (I) normoxic conditions and (J) conditions of hypoxic preconditioning or in *egl-9* mutants (see text for details). Light blue indicates low protein activity, low amounts of eicosanoids, or a defective O₂-ON response.

that CYP-13A12 and other CYPs act as acute O₂ sensors and produce eicosanoids, which are short-lived and act locally (22) during reoxygenation to signal nearby sensory circuits that drive the O₂-ON response.

In humans, a low uptake of PUFAs or an imbalanced ratio of ω 3-to- ω 6 PUFAs is associated with elevated risk of stroke, cardiovascular disease, and cancer (21, 23, 29, 30). Cytochrome P450s and eicosanoid production also have been implicated in mammalian ischemia-reperfusion (15, 21). Nonetheless, little is known concerning the causal relationships among and mechanisms relating O₂ and PUFA homeostasis, CYP, and

PUFA-mediated cell signaling and organismal susceptibility to oxidative disorders. Our results identify a pathway in which EGL-9-HIF-1 regulates CYP-eicosanoid signaling, demonstrate that PUFAs confer a rapid response to reoxygenation via CYP-generated eicosanoids, and provide direct causal links among CYPs, PUFA-derived eicosanoids, and an animal behavioral response to reoxygenation. Because the molecular mechanisms of O₂ and PUFA homeostasis are fundamentally similar and evolutionarily conserved between nematodes and mammals (7, 11, 26), we suggest that the *C. elegans* O₂-ON response is analogous to the mammalian tissue and cellular

response to ischemia-reperfusion injury and that the molecular pathway including EGL-9-HIF-1 and CYPs in controlling responses to reoxygenation after anoxia is evolutionarily conserved.

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S11

Table S1

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Robustness and Compensation of Information Transmission of Signaling Pathways

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Robust transmission of information despite the presence of variation is a fundamental problem in cellular functions. However, the capability and characteristics of information transmission in signaling pathways remain poorly understood. We describe robustness and compensation of information transmission of signaling pathways at the cell population level. We calculated the mutual information transmitted through signaling pathways for the growth factor–mediated gene expression. Growth factors appeared to carry only information sufficient for a binary decision. Information transmission was generally more robust than average signal intensity despite pharmacological perturbations, and compensation of information transmission occurred. Information transmission to the biological output of neurite extension appeared robust. Cells may use information entropy as information so that messages can be robustly transmitted despite variation in molecular activities among individual cells.

Signaling pathways transmit signals from growth factors to downstream gene expression, influencing various cell fate decisions such as cell differentiation (1). To control cellular responses by stimulation intensity, signaling pathways must reliably transmit stimulation intensity through their signaling activities. The reliability of signal transmission depends on the balance between signal intensity and variation. The smaller the signal variation, the more information can be transmitted through a pathway with the same dynamic range of signal in-

tensity. Even high-intensity signals cannot be reliably transmitted if the variation in signal intensity is large. In contrast, even signals with low intensity can be reliably transmitted if the variation in signal intensity is small (Fig. 1A). Thus, the reliability of signal transmission depends on both average (mean) intensity and variation. As a consequence, the number of controllable states of cellular responses is determined by the number of reliably transmitted signals. Intuitively, the larger the number of reliably transmitted signals, the more information the signal pathway can transmit. If cellular signaling pathways are treated as communication channels in the framework of Shannon's information theory (2–12), the amount of information that can be reliably transmitted through a cellular signaling pathway can be measured by mutual information, which corresponds to the logarithm of the average number of controllable states of a cellular response that can be defined by varied upstream signals (13–15).

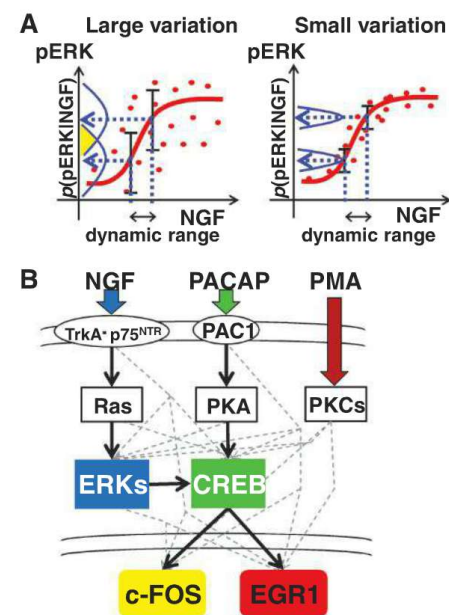


Fig. 1. Information transmission of signaling pathways. (A) Reliability of information transmission depends on both signal intensity and variation. More information can be transmitted with the same dynamic range of signal intensity if signal variation is smaller. Dots denote intensities of pERKs in individual cells, and lines denote the average intensity of pERKs. $p(\text{pERKs}|\text{NGF})$ denotes the distribution (a normalized histogram) of pERKs for a given dose of NGF. (B) Signaling pathways from growth factors, such as NGF, PACAP, and PMA to the IEGs, such as c-FOS and EGR1. Solid lines indicate the reported pathways for each growth factor, and gray dashed lines indicate other possible pathways. The colored boxes are the measured molecules, and white ovals are unmeasured molecules in this study.

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adenylate cyclase-activating peptide (PACAP), and phorbol 12-myristate 13-acetate (PMA) induce phosphorylation of extracellular signal-regulated kinase (ERK) and 3'-5'-cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) and expression of the IEGs such as c-FOS and early growth response protein 1 (EGR1), mainly through Ras-dependent, cAMP-dependent protein

kinase A (PKA)-dependent, and conventional and novel protein kinase C (PKC)-dependent signaling pathways, respectively (16–18) (Fig. 1B, solid lines). We measured the amount of phosphorylated ERK1 and ERK2 (pERKs) and CREB (pCREB) and protein abundance of c-FOS and EGR1 in the cell population (fig. S1). We used quantitative image cytometry (QIC) (19) and ob-

tained simultaneous measurements for two signaling molecules at single-cell resolution. We calculated mutual information by

$$\int dx p(x) \int dy p(y|x) \log_2 \frac{p(y|x)}{p(y)}$$

where $p(x)$ and $p(y)$ are input and output distributions, respectively, and $p(y|x)$ is a conditional distribution. For example, x and y correspond to the intensity of pCREB and c-FOS in an individual cell, and $p(x)$ and $p(y)$ correspond to the normalized histograms of pCREB and c-FOS. The conditional distribution $p(y|x)$ represents the histogram of c-FOS for a given intensity of pCREB. An information set of 1 bit means that the downstream molecules receive two distinguishable states from upstream signals. The measurement by QIC appeared reliable for calculating the joint and conditional distribution of two signaling molecules (fig. S2), as needed for the calculation of mutual information.

To explore information transmission by growth factors, we measured the mutual information between the growth factors and pCREB under conditions for which the distribution of growth factors gives the maximum mutual information between the growth factors and the IEGs (i.e., channel capacity) (Fig. 2A and fig. S3). Hereafter, we used the optimal input distribution so that mutual information between growth factors and the IEGs becomes maximum. In response to NGF and PACAP, the mutual information between growth factors and pCREB was ~1 bit (Fig. 2A, gray, fig. S3), indicating that pCREB receives the information of NGF and PACAP for a binary decision. In response to PMA, the mutual information was ~0.5 bits.

Many kinases, including ERKs and PKA, phosphorylate CREB (20). Therefore, growth factors can transmit information to pCREB through pERKs or through other pathways. We decomposed the mutual information and analyzed the contribution of pERKs for information transmission to pCREB (Fig. 2A). We defined the contribution of information transmission of a specific pathway by decomposing the mutual information, which is represented by multivariate mutual information (fig. S4), e.g., from A to B as PATHWAY:A-B. The information transmission from the growth factors (G) to pCREB (C) through pERK (E) is represented as PATHWAY:E-C (Fig. 2A, blue). The information transmission from the growth factors (G) to pCREB (C) not through pERK (E) is represented as PATHWAY:G-C (Fig. 2A, green). PATHWAY:E-C corresponds to multivariate mutual information of growth factors, pERKs, and pCREB. PATHWAY:G-C corresponds to conditional mutual information between growth factors and pCREB given pERK. The total information transmission from growth factor to pCREB is the mutual information between growth factors and pCREB, which is equal to the sum of PATHWAY:E-C and PATHWAY:G-C. Although pCREB receives similar information from NGF and PACAP, NGF mainly used PATHWAY:E-C,

Fig. 2. Information transmission of growth factors to pCREB and the IEGs. (A) Mutual information (MI) between the indicated growth factors (GF) and pCREB. MI through PATHWAY:G-C (green) and through PATHWAY:E-C (blue), and their sum, the total MI between GF and pCREB (gray). Hereafter, unless otherwise specified, MI is calculated at the time when the total MI reached a maximum. On average, ~1000 cells in response to 11 doses of NGF (0.05 to 12.15 ng/ml with exponential increase to the power of $\sqrt{3}$), 11 doses of PACAP (0.05 to 3000 nM with exponential increase to the power of 3), 11 doses of PMA (1 to 1024 ng/ml with exponential increase to the power of 2), and no stimulation were used for calculation of MI (see supplementary materials). The distribution of growth factors that gives the maximum MI (i.e., channel capacity) for c-FOS was used (see supplementary materials). A similar result was obtained when the distribution of growth factors that gives the channel capacity for EGR1 was used (fig. S3). Multivariate MI can either be positive or negative (see supplementary materials). Hereafter, the error bars indicate standard deviations estimated from 20 bootstrap sample sets in each figure. (B) MI between the growth factors and the IEGs (gray) through PATHWAY:E-I (blue), PATHWAY:C-I (green), and PATHWAY:G-I (red). c-FOS (left), EGR1 (right). The maximum of MI in the time course is shown in (A) (also see fig. S3, left column) and (B) (also see fig. S5).

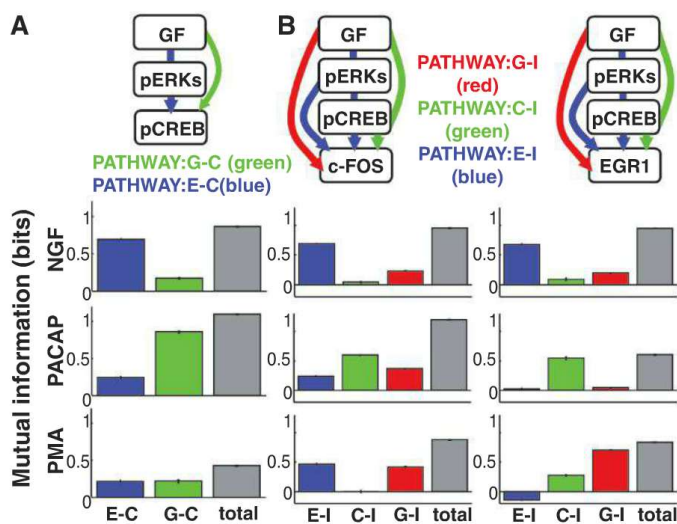
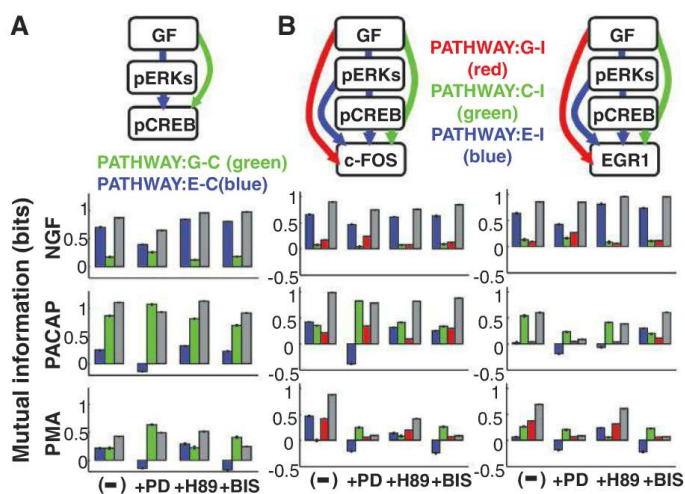


Fig. 3. Information transmission despite pharmacological perturbations. (A) MI between the indicated growth factors and pCREB (gray) through PATHWAY:G-C (blue) and PATHWAY:E-C (green) in the absence (—) or the presence of PD (5 nM PD0325901, a MEK inhibitor), H89 (5 μ M PKA inhibitor), or BIS/GF109203X (0.5 nM PKC inhibitor). Low doses of the inhibitors were used to avoid complete loss of signals and side effects (fig. S6). Here, the distribution of the indicated growth factors optimized for c-FOS in the absence of the inhibitor was used. Similar results were obtained for EGR1 (fig. S7). (B) MI between the indicated growth factors and the IEGs (gray) in the presence of the indicated inhibitor through PATHWAY:E-I (blue), PATHWAY:C-I (green), and PATHWAY:G-I (red). The MI at 50 min after stimulation is shown in (A) and (B).



whereas PACAP used PATHWAY:G-C for information transmission to pCREB (Fig. 2A). These results indicate that, for information transmission to pCREB, NGF mainly uses pERKs-dependent pathways and PACAP mainly uses pERKs-independent pathways, probably the PKA pathway.

We also measured the mutual information between growth factors and the IEGs (Fig. 2B and fig. S5). In response to NGF, the mutual information between NGF and the IEGs was ~ 1 bit, indicating that the IEGs receive enough information from NGF for a binary decision (Fig. 2B). The mutual information between PACAP and the transcriptional factor c-FOS was also ~ 1 bit, whereas that between PACAP and EGR1 was ~ 0.5 bits, indicating that only c-FOS receives enough information from PACAP for a binary decision.

We decomposed information (fig. S4) transmitted from growth factor through pERK to the IEG (PATHWAY:E-I) (Fig. 2B, blue), from growth factor through pCREB not through pERK to the IEG (PATHWAY:C-I) (Fig. 2B, green), and from growth factor not through pERK and pCREB to the IEG (PATHWAY:G-I) (Fig. 2B, red). In general, mutual information is non-negative, but multivariate mutual information of more than three variables can be negative (fig. S4). NGF used PATHWAY:E-C as the main pathway for information transmission to pCREB (Fig. 2A, blue) and PATHWAY:E-I as the main pathway to the IEGs (Fig. 2B, blue). PACAP used PATHWAY:G-C as the main pathway for information transmission to pCREB (Fig. 2A, green) and PATHWAY:C-I as the main pathway to the IEGs (Fig. 2B, green).

PMA used PATHWAY:G-I as the main pathway to EGR1 (Fig. 2B, red). We identified the main pathway for information transmission to the IEGs solely by measuring cell populations, without conventional pharmacological perturbation. We also calculated the contribution of unmeasured molecules, which can not be calculated by conventional methods using only averaged intensity. For example, the contribution of pERK3 (21, 22), which was not recognized by the antibody used in this study, to information transmission to pCREB was implicitly included in PATHWAY:G-C (Fig. 2A, green). Similarly, the contributions of NF κ B (nuclear factor κ B) (23)—which is activated in response to the p75 neurotrophin receptor (p75^{NTR}, a low-affinity receptor for NGF)—to information transmission to the IEGs was implicitly included in PATHWAY:G-I (Fig. 2B, red). PMA also activates multiple isoforms of PKCs (16). Contributions of PKCs were also included in PATHWAY:G-I (Fig. 2B, red). Given that the mutual information between PMA and the IEGs was larger than that between PMA and pCREB, PMA appears to use pathways other than pCREB, probably PKCs, for information transmission to the IEGs. Consequently, we conclude that NGF, PACAP, and PMA use different pathways to transmit information to the IEGs for a binary decision.

We pharmacologically perturbed specific pathways by the addition of inhibitors and examined the effects on information transmission (Fig. 3 and fig. S6). PD0325901, MAPK/ERK kinase (MEK) inhibitor that inhibits activation of pERKs, decreased information transmission from growth

factors to pCREB through PATHWAY:E-C (Fig. 3A). However, in response to PACAP and PMA, information transmission through PATHWAY:G-C increased in cells treated with PD0325901 and compensated for the decreased information transmission through PATHWAY:E-C, allowing robust information transmission from growth factors to pCREB. H89, a PKA inhibitor, did not affect information transmission to pCREB, although the average intensity of pCREB was decreased by the inhibitor (Fig. 3A and fig. S6). Thus, mutual information appears to be more robust than average intensity, despite some specific perturbations. Addition of bisindolylmaleimide (BIS/GF109203X), a PKC inhibitor, did not affect information transmission from NGF or PACAP to pCREB through PATHWAY:E-C but decreased information transmission from PMA through PATHWAY:E-C. Thus, there appears to be cross-talk between the PKCs and ERK pathways (24). Information transmission from NGF to the IEGs and from PACAP to c-FOS remained at ~ 1 bit despite the pharmacological perturbations (Fig. 3B), indicating robust information transmission in these pathways. In the presence of PD0325901, information transmission to the IEGs through PATHWAY:E-I decreased, whereas that through other pathways compensatorily increased. In contrast, H89 and BIS did not strongly affect information transmission through any pathway. Pharmacological perturbation allowed us to analyze the robustness and compensation of information transmission to perturbation. Although inhibitors can inhibit other kinases besides their main targets (25, 26), such additional side effects of the inhibitors were im-

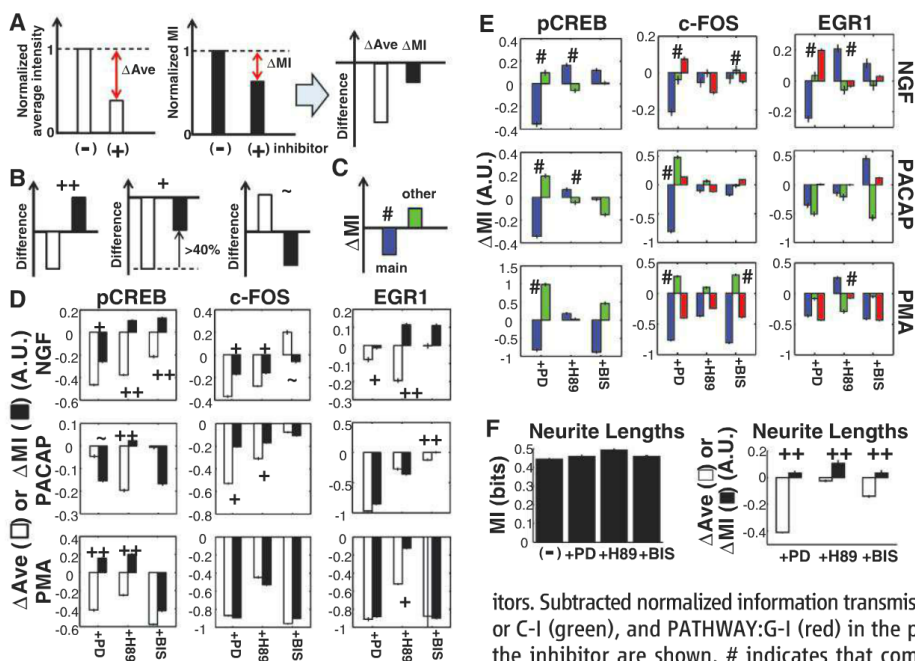


Fig. 4. Robustness and compensation of information transmission. (A) Δ Ave (open bar) and Δ MI (closed bar) were defined by subtracting normalized average intensity and MI in the absence of the inhibitor from those in the presence of the inhibitor. (B) Examples of ++ (negative Δ Ave and positive Δ MI; MI is robust), + (negative Δ MI is 40% bigger than negative Δ Ave; MI is weakly robust), and ~ (positive Δ Ave and negative Δ MI; MI is not robust) in (A) and (F) are shown. (C) Main and Other were the subtracted MI of the main and other pathways in the absence of the inhibitor, respectively. The combinations of decrease of information transmission through the main pathway and increase of information transmission through other pathways are indicated by #. (D) Δ Ave (open bar) and Δ MI (closed bar) in the presence of the inhibitor subtracted from those in the absence of the inhibitor are shown. (E) Compensation of information transmission through the indicated pathways in the presence of the inhibitors. Subtracted normalized information transmission through PATHWAYS:E-C or E-I (blue), PATHWAYS:G-C or C-I (green), and PATHWAY:G-I (red) in the presence of the inhibitor from those in the absence of the inhibitor are shown. # indicates that compensation occurred. (F) Information transmission to NGF-dependent neurite lengths at 24 hours after stimulation in the presence or absence of the inhibitors. The optimal distribution of NGF in the absence of the inhibitor was used for calculation of MI. Robustness of information transmission to neurite extension in the presence of the indicated inhibitors (right panel). ++ indicates the negative Δ Ave (open bar) and positive Δ MI (closed bar); MI is robust.

implicitly included in one of the pathways, such as PATHWAY:E-C and PATHWAY:G-C (Fig. 3 and supplementary text).

We further examined the robustness of information transmission by comparing the effect of the inhibitors on the average intensity of the signal and mutual information (Fig. 4A, B, and D and fig. S6). Pharmacological perturbations that decreased the average intensity without changes in the shape of distribution also decrease the mutual information. However, despite decreased average intensity, in some cases, mutual information such as that to pCREB in response to NGF in the presence of H89 actually increased (Fig. 4D, ++). In some cases, mutual information such as that to pCREB in response to NGF in cells treated with PD0325901 (or that to c-FOS in response to NGF in cells exposed to PD0325901 or H89) decreased less than the average intensity of the signal (Fig. 4D, +). Only a few pathways showed a greater decrease of mutual information than that of average intensity (Fig. 4D, ~). These results indicate that mutual information is generally more robust than average intensity in the presence of the tested perturbations.

We examined compensation in information transmission (Fig. 4, C and E). Treatment of cells with PD0325901 decreased information transmission from NGF to pCREB and the IEGs through PATHWAY:E-C and PATHWAY:E-I but increased information transmission through PATHWAY:G-C and PATHWAYS:C-I or G-I. This indicates compensation through other pathways in the signaling pathway. Such compensation was also seen in information transmission from NGF to pCREB and the IEGs, from PACAP to pCREB and c-FOS, and from PMA to pCREB and the IEGs (Fig. 4E, #). Both robustness and compensation occurred concomitantly in some cases, such as information transmission from NGF to pCREB, c-FOS, and EGR1 in the presence of PD0325901 (Fig. 4D and E), suggesting that compensation may increase robustness in these pathways. However, only compensation, but not robustness, was seen in some cases, such as information transmission from PACAP to pCREB in the presence of PD0325901 and from PMA to c-FOS in the presence of PD0325901 or BIS. Increased information transmission through the other pathway apparently does not always compensate for the decrease in information carried by the main pathway. Only robustness, but not compensation, was seen in some cases, such as information transmission from PACAP to EGR1 in the presence of BIS. Therefore, the compensation does not always make robustness, and the robustness is not always caused by compensation. For the pathways that were robust despite perturbations (Fig. 4D), the mutual information with respect to the average intensity was hyperbolic and saturated at a high average intensity (fig. S8), indicating that mutual information was less sensitive to the changes in concentration of the growth factors than was average intensity. This may be one of the reasons for the robustness of information

transmission to perturbation, and the mechanism of robustness was analyzed by a toy model (figs. S9 to S14).

We investigated whether information transmission to a final biological output—neurite extension—was similarly robust to perturbation (Fig. 4F and fig. S15). Mutual information between NGF and neurite lengths were 0.5 bits in the presence or absence of the inhibitors. Mutual information between growth factors and neurite lengths, the final biological output, was also more robust than the average intensity of neurite lengths under perturbation, as was the case for the mutual information between growth factors and signaling molecules. Thus, although neurite lengths were reduced by the inhibitors, precision of neurite length control was retained. The effects of each inhibitor on the mutual information and average intensity of neurite lengths were similar to those on signaling from NGF to pCREB and to EGR1 rather than signaling to c-FOS. Thus, information on precise control of neurite length appears to be transmitted by pCREB and EGR1 rather than c-FOS, although both EGR1 and c-FOS regulate NGF-dependent neurite extension in PC12 cells (27, 28). Robustness of the mutual information between growth factors and neurite length to perturbation can be interpreted to imply that the average number of controllable states of the population of neurite lengths controlled by growth factors can be maintained despite changes in average neurite lengths caused by perturbation. The property of independence of absolute chemical concentration for information transmission to a biological phenotype may be advantageous as a design principle of body planning independent of body size. Otherwise, as the body size increased, higher concentration of growth factors would be required to maintain the same precision.

Mutual information was calculated from the distribution at each time point in this study. Ideally, mutual information of the transient response should be calculated from the distribution of the temporal trajectories of the input and output molecule activities (4, 8, 10, 29). Mutual information between input and output is always decreased compared with that between input and intermediate, that is, data-processing inequality holds. The inequality seems to be useful to understand the information transmission of the signaling network (supplementary text).

We found the robustness and compensation of signaling pathways at the cell population level (fig. S16). Our results indicate that robust signaling pathways that use information entropy as information are more robust to noise than is signal intensity. Despite the variation of signal intensity and abundance of molecules between individual cells, cells can reliably receive growth factor information through robust signaling pathways.

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Supplementary Materials

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Sequencing Y Chromosomes Resolves Discrepancy in Time to Common Ancestor of Males Versus Females

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The Y chromosome and the mitochondrial genome have been used to estimate when the common patrilineal and matrilineal ancestors of humans lived. We sequenced the genomes of 69 males from nine populations, including two in which we find basal branches of the Y-chromosome tree. We identify ancient phylogenetic structure within African haplogroups and resolve a long-standing ambiguity deep within the tree. Applying equivalent methodologies to the Y chromosome and the mitochondrial genome, we estimate the time to the most recent common ancestor (T_{MRC}) of the Y chromosome to be 120 to 156 thousand years and the mitochondrial genome T_{MRC} to be 99 to 148 thousand years. Our findings suggest that, contrary to previous claims, male lineages do not coalesce significantly more recently than female lineages.

The Y chromosome contains the longest stretch of nonrecombining DNA in the human genome and is therefore a powerful tool with which to study human history. Estimates of the time to the most recent common ancestor (T_{MRC}) of the Y chromosome have differed by a factor of about 2 from T_{MRC} estimates for the mitochondrial genome. Y-chromosome coalescence time has been estimated in the range of 50 to 115 thousand years (ky) (1–3), although larger values have been reported (4, 5), whereas estimates for mitochondrial DNA (mtDNA) range from 150 to 240 ky (3, 6, 7). However, the quality and quantity of data available for these two uniparental loci have differed substantially. Whereas

the complete mitochondrial genome has been resequenced thousands of times (6, 8), fully sequenced diverse Y chromosomes have only recently become available. Previous estimates of the Y-chromosome T_{MRC} relied on short resequenced segments, rapidly mutating microsatellites, or single-nucleotide polymorphisms (SNPs) ascertained in a small panel of individuals and then genotyped in a global panel. These approaches likely underestimate genetic diversity and, consequently, T_{MRC} (9).

We sequenced the complete Y chromosomes of 69 males from seven globally diverse populations of the Human Genome Diversity Panel (HGDP) and two additional African populations:

San (Bushmen) from Namibia, Mbuti Pygmies from the Democratic Republic of Congo, Baka Pygmies and Nzebi from Gabon, Mozabite Berbers from Algeria, Pashtuns (Pathan) from Pakistan, Cambodians, Yakut from Siberia, and Mayans from Mexico (fig. S1). Individuals were selected without regard to their Y-chromosome haplogroups.

The Y-chromosome reference sequence is 59.36 Mb, but this includes a 30-Mb stretch of constitutive heterochromatin on the q arm, a 3-Mb centromere, 2.65-Mb and 330-kb telomeric pseudoautosomal regions (PAR) that recombine with the X chromosome, and eight smaller gaps. We mapped reads to the remaining 22.98 Mb of assembled reference sequence, which consists of three sequence classes defined by their complexity and degree of homology to the X chromosome (10): X-degenerate, X-transposed, and ampliconic. Both the high degree of self-identity within the ampliconic tracts and the X-chromosome homology of the X-transposed region render portions of the Y chromosome ill suited for short-read sequencing. To address this, we constructed filters that reduced the data to 9.99 million sites (11)

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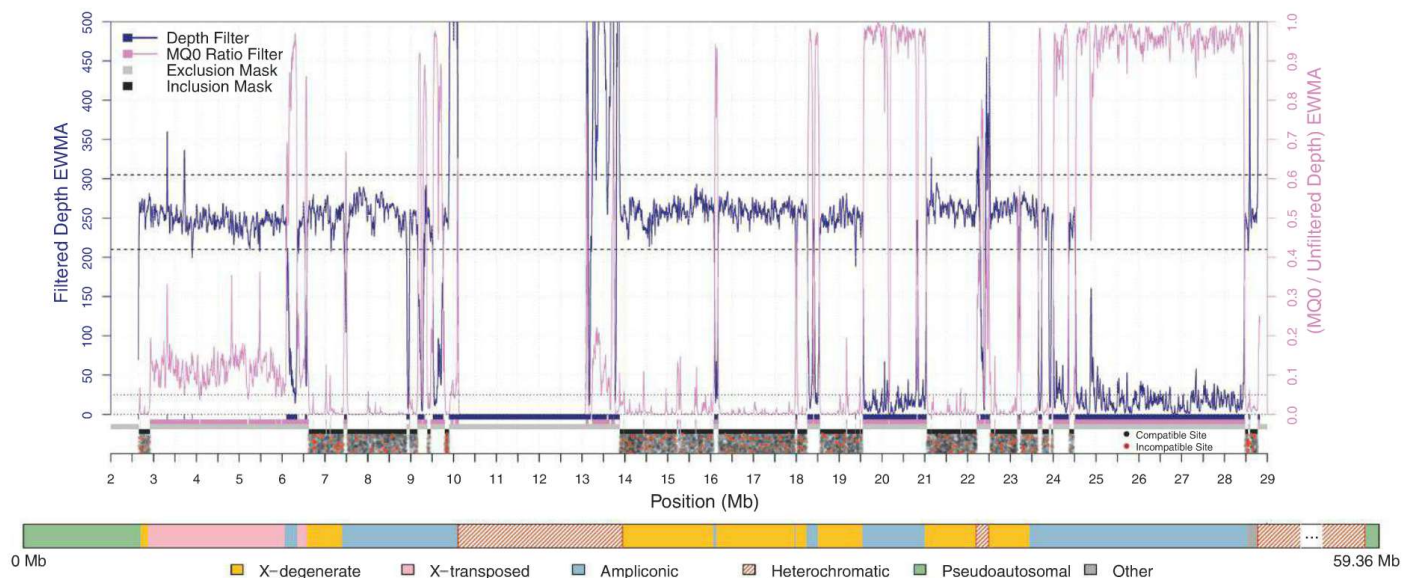


Fig. 1. Callability mask for the Y chromosome. Exponentially weighted moving averages of read depth (blue line) and the proportion of reads mapping ambiguously (MQ0 ratio; violet line) versus physical position. Regions with values outside the envelopes defined by the dashed lines (depth) or dotted lines (MQ0) were flagged (blue and violet boxes) and merged for exclusion (gray boxes). The complement (black boxes) defines

the regions within which reliable genotype calls can be made. Below, a scatter plot indicates the positions of all observed SNVs. Those incompatible with the inferred phylogenetic tree (red) are uniformly distributed. The X-degenerate regions yield quality sequence data, ampliconic sequences tend to fail both filters, and mapping quality is poor in the X-transposed region.

(Fig. 1 and fig. S2). We then implemented a haploid model expectation-maximization algorithm to call genotypes (11).

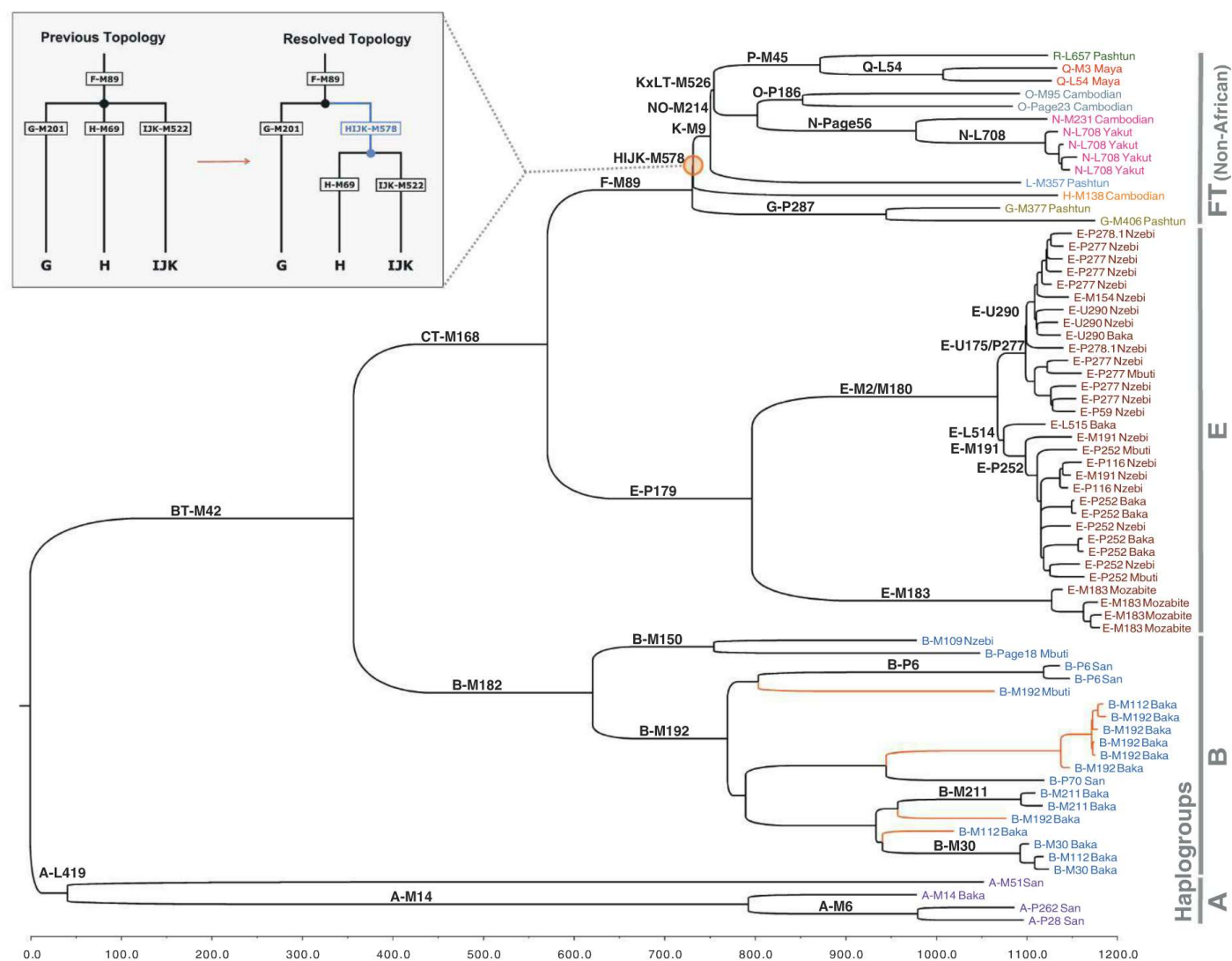
We identified 11,640 single-nucleotide variants (SNVs) (fig. S3). A total of 2293 (19.7%) are present in dbSNP (v135), and we assigned haplogroups on the basis of the 390 (3.4%) present in the International Society of Genetic Genealogy (ISOGG) database (12) (fig. S4). At SNVs, median haploid coverage was 3.1x (interquartile range 2.6 to 3.8x) (table S1 and fig. S5), and sequence validation suggests a genotype calling error rate on the order of 0.1% (11).

Because mutations accumulate over time along a single lengthy haplotype (13), the male-specific region of the Y chromosome provides power for phylogenetic inference. We constructed a maximum likelihood tree from 11,640 SNVs using the Tamura-Nei nucleotide substitution

model (Fig. 2) and, in agreement with (14), observe strong bootstrap support (500 replicates) for the major haplogroup branching points. The tree both recapitulates and adds resolution to the previously inferred Y-chromosome phylogeny (fig. S6), and it characterizes branch lengths free of ascertainment bias. We identify extraordinary depth within Africa, including lineages sampled from the San hunter-gatherers that coalesce just short of the root of the entire tree. This stands in contrast to a tree from autosomal SNP genotypes (15), wherein African branches were considerably shorter than others; genotyping arrays primarily rely on SNPs ascertained in European populations and therefore undersample diversity within Africa. Two regions of reduced branch length in our tree correspond to rapid expansions: the out-of-Africa event (downstream of F-M89) and the agriculture-catalyzed Bantu

expansions (downstream of E-M2). Among the three hunter-gatherer populations, we find a relatively high number of B2 lineages. Within this haplogroup, six Baka B-M192 individuals form a distinct clade that does not correspond to extant definitions (11) (fig. S7). We estimate this previously uncharacterized structure to have arisen ~35 thousand years ago (kya).

We resolve the polytomy of the Y macrohaplogroup F (16) by determining the branching order of haplogroups G, H, and IJK (Fig. 2 and fig. S6). We identified a single variant (rs73614810, a C→T transition dubbed “M578”) for which haplogroup G retains the ancestral allele, whereas its brother clades (H and IJK) share the derived allele. Genotyping M578 in a diverse panel confirmed the finding (table S2). We thereby infer more recent common ancestry between hgH and hgIJK than between either and hgG. M578 de-



finds an early diversification episode of the Y phylogeny in Eurasia (11).

To account for missing genotypes, we assigned each SNV to the root of the smallest subtree containing all carriers of one allele or the other and inferred that the allele specific to the subtree was derived (fig. S8). We used the chimpanzee Y-chromosome sequence to polarize 398 variants assigned to the deepest split—a task complicated by substantial structural divergence (11, 17).

We estimated the coalescence time of all Y chromosomes using both a molecular clock–based frequentist estimator and an empirical Bayes approach that uses a prior distribution of T_{MRCA} from coalescent theory and conducts Markov chain simulation to estimate the likelihood of parameters given a set of DNA sequences (GENETREE) (11, 18) (Table 1). To directly compare the T_{MRCA} of the Y chromosome to that of the mtDNA, we estimated their respective mutation rates by calibrating phylogeographic patterns from the initial peopling of the Americas, a recent human event with high-confidence archaeological dating.

Archaeological evidence indicates that humans first colonized the Americas ~15 kya via a rapid coastal migration that reached Monte Verde II in southern Chile by 14.6 kya (19). The two Native American Mayans represent Y-chromosome hgQ lineages, Q-M3 and Q-L54*(xM3), that likely diverged at about the same time as the initial peopling of the continents. Q is defined by the M242 mutation that arose in Asia. A descendent haplogroup, Q-L54, emerged in Siberia and is ancestral to Q-M3. Because the M3 mutation appears to be specific to the Americas (20), it likely occurred after the initial entry, and the prevalence of M3 in South America suggests that it emerged before the southward migratory wave. Consequently, the divergence between these two lineages provides an appropriate calibration point for the Y mutation rate. The large number of variants that have accumulated since divergence, 120 and 126, contrasts with the pedigree-based estimate of the Y-chromosome mutation rate, which is based on just 4 mutations (21). Using entry to the Americas as a calibration point, we estimate a mutation rate of 0.82×10^{-9} per base pair (bp) per year [95% confidence interval (CI): 0.72×10^{-9} to 0.92×10^{-9} /bp/year] (table S3). False negatives have minimal effect on this estimate due to the low probability, at 5.7x and 8.5x coverage, of observing fewer than two reads at a site (observed proportions: 3.1% and 0.6%) and due to the fact that the number of unobserved singletons possessed by one individual is offset by a similar number of Q doubletons unobserved in the same individual and thereby misclassified as singletons possessed by the other (11) (figs. S9 and S10). This calibration approach assumes approximate coincidence between the expansion throughout the Americas and the divergence of Q-M3 and Q-L54*(xM3), but we consider deviation from this assumption and identify a strict lower bound on the point of

divergence using sequences from the 1000 Genomes Project (11). As a comparison point, we consider the out-of-Africa expansion of modern humans, which dates to approximately 50 kya (22) and yields a similar mutation rate of 0.79×10^{-9} /bp/year.

We constructed an analogous pipeline for high coverage (>250x) mtDNA sequences from the 69 male samples and an additional 24 females from the seven HGDP populations (11) (fig. S11). As in the Y-chromosome analysis, we calibrated the mtDNA mutation rate using divergence within the Americas. We selected the pan-American hgA2, one of several initial founding haplogroups among Native Americans. The star-shaped phylogeny of hgA2 subclades suggests that its divergence was coincident with the rapid dispersal upon the initial colonization of the continents (23). Calibration on 108 previously analyzed hgA2 sequences (11) (fig. S12) yields a point estimate equivalent to that from our seven Mayan mtDNAs, but within a narrower confidence interval. From this within-human calibration, we estimate a mutation rate of 2.3×10^{-8} /bp/year (95% CI: 2.0×10^{-8} to 2.5×10^{-8} /bp/year), higher than that from human-chimpanzee divergence but similar to other estimates using within-human calibration points (24, 25).

The global T_{MRCA} estimate for any locus constitutes an upper bound for the time of human

population divergence under models without gene flow. We estimate the Y-chromosome T_{MRCA} to be 138 ky (120 to 156 ky) and the mtDNA T_{MRCA} to be 124 ky (99 to 148 ky) (Table 1) (11). Our mtDNA estimate is more recent than many previous studies, the majority of which used mutation rates extrapolated from between-species divergence. However, mtDNA mutation rates are subject to a time-dependent decline, with pedigree-based estimates on the faster end of the spectrum and species-based estimates on the slower. Because of this time dependency and the need to calibrate the Y and mtDNA in a comparable manner, it is more appropriate here to use within-human clade estimates of the mutation rate.

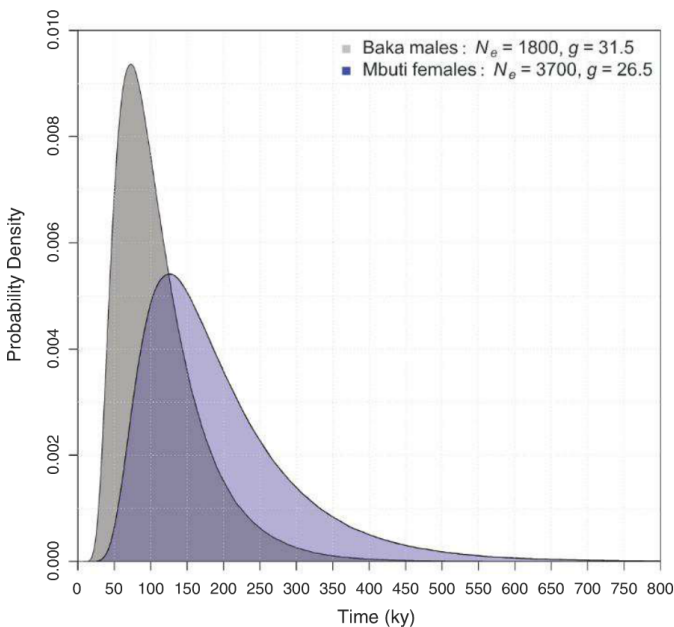
Rather than assume the mutation rate to be a known constant, we explicitly account for the uncertainty in its estimation by modeling each T_{MRCA} as the ratio of two random variables. We estimate the ratio of the mtDNA T_{MRCA} to that of the Y chromosome to be 0.90 (95% CI: 0.68 to 1.11) (fig. S13). If, as argued above, the divergence of the Y-chromosome Q lineages occurred at approximately the same time as that of the mtDNA A2 lineages, then the T_{MRCA} ratio is invariant to the specific calibration time used. Regardless, the conclusion of parity is robust to possible discrepancy between the divergence times within the Americas (11). Using comparable calibration approaches, the Y and

Table 1. T_{MRCA} and N_e estimates for the Y chromosome and mtDNA. Pop., population.

Method	Y chromosome				mtDNA			
	Pop.	n	T_{MRCA}^*	N_e	Pop.	n	T_{MRCA}^*	N_e
Molecular clock	All	69	139 (120–156)	4500 [†]	All	93	124 (99–148)	9500 [†]
GENETREE [‡]	San	6	128 (112–146)	3800	Nzebi	18	105 (91–119)	11,500
	Baka	11	122 (106–137)	1800	Mbuti	6	121 (100–143)	3700

*Employs mutation rate estimated from within-human calibration point. Times measured in ky. †Uses Watterson's estimator, θ_w . ‡Each coalescent analysis restricted to a single population spanning the ancestral root (11).

Fig. 3. Similarity of T_{MRCA} does not imply equivalent N_e of males and females. The T_{MRCA} for a given locus is drawn from a predata (i.e., prior) distribution that is a function of N_e , generation time, sample size, and demographic history. Consider the distribution of possible T_{MRCA} s for a set of 100 uniparental chromosomes. Although the Mbuti mtDNA N_e is twice as large as that of the Baka Y chromosome, the corresponding predata T_{MRCA} distributions overlap considerably.



mtDNA coalescence times are not significantly different. This conclusion would hold whether or not an alternative approach would yield more definitive T_{MRCA} estimates.

Our observation that the T_{MRCA} of the Y chromosome is similar to that of the mtDNA does not imply that the effective population sizes (N_e) of males and females are similar. In fact, we observe a larger N_e in females than in males (Table 1). Although, due to its larger N_e , the distribution from which the mitochondrial T_{MRCA} has been drawn is right-shifted with respect to that of the Y-chromosome T_{MRCA} , the two distributions have large variances and overlap (Fig. 3).

Dogma has held that the common ancestor of human patrilineal lineages, popularly referred to as the Y-chromosome “Adam,” lived considerably more recently than the common ancestor of female lineages, the so-called mitochondrial “Eve.” However, we conclude that the mitochondrial coalescence time is not substantially greater than that of the Y chromosome. Indeed, due to our moderate-coverage sequencing and the existence of additional rare divergent haplogroups, our analysis may yet underestimate the true Y-chromosome T_{MRCA} .

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Supplementary Materials

www.sciencemag.org/cgi/content/full/341/6145/562/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S13

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Data File S1

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Low-Pass DNA Sequencing of 1200 Sardinians Reconstructs European Y-Chromosome Phylogeny

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Genetic variation within the male-specific portion of the Y chromosome (MSY) can clarify the origins of contemporary populations, but previous studies were hampered by partial genetic information. Population sequencing of 1204 Sardinian males identified 11,763 MSY single-nucleotide polymorphisms, 6751 of which have not previously been observed. We constructed a MSY phylogenetic tree containing all main haplogroups found in Europe, along with many Sardinian-specific lineage clusters within each haplogroup. The tree was calibrated with archaeological data from the initial expansion of the Sardinian population ~7700 years ago. The ages of nodes highlight different genetic strata in Sardinia and reveal the presumptive timing of coalescence with other human populations. We calculate a putative age for coalescence of ~180,000 to 200,000 years ago, which is consistent with previous mitochondrial DNA-based estimates.

New sequencing technologies have provided genomic data sets that can reconstruct past events in human evolution

more accurately (1). Sequencing data from the male-specific portion of the Y chromosome (MSY) (2), because of its lack of recombination and low

mutation, reversion, and recurrence rates, can be particularly informative for these evolutionary analyses (3, 4). Recently, high-coverage Y chromosome sequencing data from 36 males from different worldwide populations (5) assessed 6662 phylogenetically informative variants and estimated the timing of past events, including a putative coalescence time for modern humans of ~101,000 to 115,000 years ago.

MSY sequencing data reported to date still represent a relatively small number of individuals from a few populations. Furthermore, dating estimates are also affected by the calibration of the

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†Laura Morelli prematurely passed away on 20 February 2013. This work is dedicated to her memory.

phylogenetic tree used to establish the rate of molecular change over time. This calibration can either correlate the number of nucleotide substitutions with dates from paleontological/archaeological records (phylogenetic rate) or can use directly observed de novo mutations in present-day families (mutation rate). However, both approaches are complicated by several variables (6, 7).

Some of these problems can be resolved by the analysis of MSY sequencing data from many individuals from a genetically informative population, regarding which archaeological data are available to provide suitable calibration points. This prompted us to use large-scale MSY sequencing data from the island population of Sardinia for phylogenetic analysis. We generated a high-resolution analysis of the MSY from population sequencing of 1204 Sardinian males (8). We used a hierarchical approach and, to be consistent with previous work (5), focused on approximately 8.97 megabase pairs (Mbp) from the Y chromosome in the X-degenerated region. We inferred 11,763 MSY phylogenetically informative single-nucleotide polymorphisms (SNPs), detected in at least two individuals and unequivocally associated with specific haplogroups and sub-haplogroups; 6751 of these SNPs had not thus far been reported in public databases.

The informative SNPs were used to construct a parsimony-based phylogenetic tree. To root the tree, we used the chimpanzee genome reference as an outgroup and inferred the ancestral status at all SNP sites except for 26 that were discarded in further analysis. The first bifurcation point, and thus the most recent common ancestor, separates samples 1 to 7 from the rest of the samples (samples 8 to 1204) (Table 1). The average number of derived alleles in the 1204 males is 1002.6 (± 21.2 SD) which, consistent with a neutral evolution of these Y polymorphisms, shows a remarkable uniformity of the branch length.

The Sardinian sequences show a very high degree of inter-individual variation. As shown in a schematic tree (Fig. 1), all of the most common Y-chromosome haplogroups previously detected in Europe are present in our sample (Table 1), with the sole exception of the northernmost Uralic haplogroup N. The first bifurcation separates the mostly sub-Saharan haplogroup A (7 individuals, 0.6% in our sample) from the others. Haplogroup E (132, 11.0%) is present with its European clade, characterized by the presence of the M35 marker, together with a small number of individuals belonging to the mainly African clade E1a. The rare haplogroup F (7, 0.6%) is related to haplogroup G (131, 10.9%), which shows a private Sardinian-Corsican clade whose ancient roots have been found in an Eneolithic sample from the Italian Alps (9). Haplogroup I (490, 40.7%) is of special interest because it is mostly represented by the I2a1a clade, identified by the M26 marker, which is at high frequencies in Sardinia (10) but is rare or absent elsewhere (11). Haplogroup J (161, 13.4%) is observed with its main subgroups; and the super-haplogroup K is present with the re-

lated L and T branches (36, 3.0%), with a single individual of haplogroup Q (1, 0.08%) and with the more common haplogroup R (239, 19.9%) occurring mostly as the western European M173-M269 branch.

Almost half of the discovered SNPs (4872) make up the skeleton of the phylogenetic tree and constitute the root of the main clades. The skeleton includes lineages that are unbranched for most of their length, with ramifications only in the terminal portion. This indicates an early separation of the clades, followed by new variability generated during subsequent expansion events.

To estimate points of divergence between Sardinian and continental clades, we sequenced two samples from the Basque Country and northern Italy, belonging to haplogroup I, and two, from Tuscany and Corsica, belonging to haplogroup G. We also analyzed the sequence of the so-called Iceman Ötzi (9), together with 133 publicly available European sequences from the 1000 Genomes Project database and those SNPs from the International Society of Genetic Genealogy (ISOGG) database detected outside Sardinia.

The Basque individual separates from the basal position of the I2a1a branch that encompasses

Table 1. Super-haplogroups, haplogroups, sub-haplogroups, and private Sardinian-Corsican clades. Here the average number of SNPs defining each class is shown in our 1209 samples. The asterisk (*) denotes that the average number of SNPs for haplogroups A and E cannot be determined with precision because of the lack in our sample of individuals belonging to haplogroups B, C, and D. Consequently, the number reported here is an overestimate. The Sardinian samples are progressively numbered from 1 to 1204, and the non-Sardinian samples are labeled as follows: O, Ötzi; T, Tuscan; B, Basque; C, Corsican; I, northern Italian. The clades containing only private Sardinian SNPs are indicated in Greek letters (progressively from α to δ within each haplogroup).

Super-haplogroup (individual no.)	Mean SNPs	Haplogroup (individual no.)	Mean SNPs	Sub-haplogroup (individual no.)	Mean SNPs	Private Sardinian clade (individual no.)	Mean SNPs
A-R (1–1204; OTCBI)	1002.6	A (1–7)	879.9*	A1b1b2b (1–7)	11.9	α (1–7)	11.9
				E1a1 (8–13)	7.0	α (8–13)	7.0
				E1b1b1a1 (14–45)	87.4		
E-R (8–1204; OTCBI)		E (8–139)	541.8*	E1b1b1b1 (46–115)	96.1	β (49–115)	15.6
				E1b1b1b2 (116–139)	114.9	γ (116–131)	25.8
		F (140–146)	299.0	F3 (140–146)	79.3		
F-R (140–1204; OTCBI)	534.8	G (147–277; OTC)	373.8	G2a2b (147–186; OTC)	109.5	α (C; 155–162)	42.8
				G2a3 (187–277)	120.3	β (163–186)	29.4
				I1a3a2 (278–279)	0.0	γ (247–277)	25.0
						α (280–285)	36.0
						β (286–296)	39.1
						γ (297–314)	34.1
I-J (278–928; BI)	387.0	I (278–767; BI)	353.5	I2a1a (280–744; BI)	106.2	δ (315–744)	37.3
				I2a1b (745–746)	0.0		
				I2a2a (747–756)	38.1		
				I2c (757–767)	72.2		
				J1c (768–830)	112.7	α (816–830)	11.0
				J2a (831–905)	125.1		
K-R (929–1204)	375.3	J (768–928)	334.3	J2b (906–928)	91.9		
				L (929–936)	123.7		
		K (929–964)	324.9	T (937–964)	101.3		
				Q1a3c (965)	0.0		
		P (965–1204)	359.1	R1a1a1 (966–980)	13.8		
				R1b1a2 (981–1165)	37.2	α (981–989)	23.0
		R (966–1204)	241.2	R1b1c (1166–1194)	75.7	β (991–1165)	29.4
				R2a1 (1195–1204)	8.5	γ (1177–1194)	36.2
						δ (1195–1204)	8.5

11 Sardinian individuals. The northern Italian sample, instead, most likely reflecting the last step of I2a1 lineages before their arrival in Sardinia, is at the basal point of most of the remaining I2a1a samples (Fig. 2). Considering two other basal lineages encompassing only Sardinian samples, we can infer that when the I2a1a sub-haplogroup entered Sardinia, it had already differentiated into four founder lineages that then accumulated private Sardinian variability. Two other founder clades show similar divergence after entry into the island: one belonging to haplogroup R1b1c (xV35) (whose differentiation is identified contrasting the Sardinian data with the ISOGG and 1000 Genome data), and the other to haplogroup G2a2b-L166 (identified by divergence from a sequenced Corsican sample).

The branch length uniformity observed in our phylogeny is consistent (Fig. 1) with a relative-

ly constant accumulation of SNPs in different lineages over time. Hence, this accumulation can be effectively used as a molecular clock for the dating of branch points. We calibrated the accumulation of Sardinian-specific genetic variation against established Sardinian archaeological records indicating a putative age of initial demographic expansion ~7700 years ago [reviewed in figs. S7 and S8 and the supplementary text (8, 12)] that is also supported by mitochondrial DNA (mtDNA) analyses (13). Comparison of Sardinian genetic variation with that found elsewhere helped us to establish the amount of variability produced during and after this expansion, resulting in sublineages that appear to be unique to the island.

We focused our calibration analyses on the individuals belonging to the I2a1a- δ clade, which

is shared by 435 individuals and is best suited to assess the Sardinian specific variability. Taking into account the average variation of all Sardinian individuals in the common I2a1a- δ clade of 37.3 (± 7.8) SNPs, a calibration point of 7700 years ago results in a phylogenetic rate of one new mutation every 205 (± 50) years. Considering that our analysis focused on approximately 8.97 Mbp of sequence from the Y chromosome X-degenerated region, this rate is equivalent to $0.53 \times 10^{-9} \text{ bp}^{-1} \text{ year}^{-1}$. This phylogenetic rate is consistent with the value of 0.617 (0.439 to $0.707 \times 10^{-9} \text{ bp}^{-1} \text{ year}^{-1}$) from the genome-wide mutation rate observed from de novo mutations adjusted for Y chromosome-specific variables (14). Our mutation rate is instead lower than the value of $1.0 \times 10^{-9} \text{ bp}^{-1} \text{ year}^{-1}$ obtained from de novo MSY mutations in a single deep-rooted

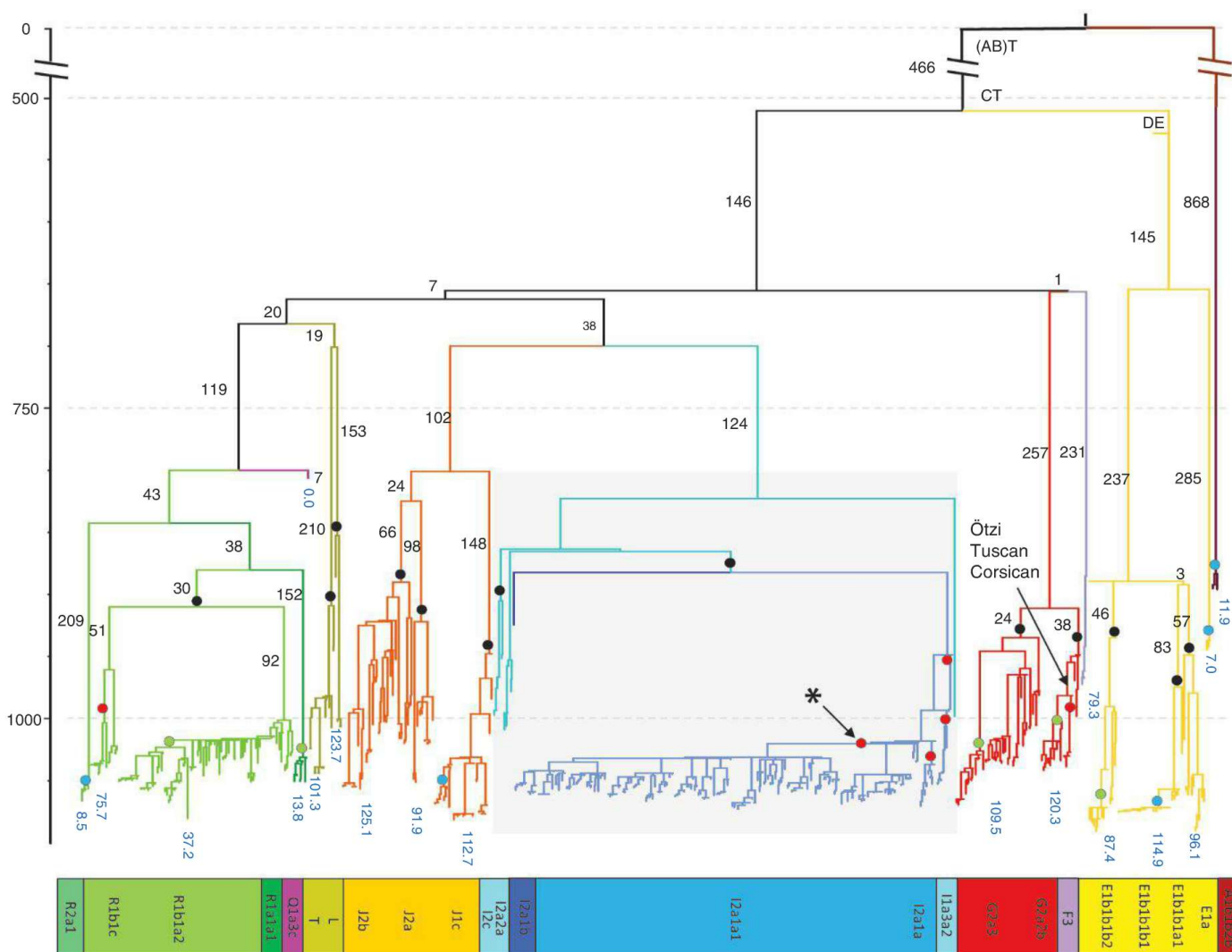


Fig. 1. Phylogenetic tree of the 1209 (1204 Sardinians and 5 non-Sardinians) Y-chromosome sequences. The bifurcations AT, BT, CT, and DE have been inferred because of the absence of individuals belonging to haplogroups B, C, and D in our sample. Colored branches represent different Y-chromosome haplotypes. The number of polymorphisms for the main branches is shown in black; the average number of SNPs of sub-haplogroups is given in blue. The sub-haplogroups are named according to ISOGG no-

menclature. The left axis indicates the number of SNPs from the root. The asterisk indicates the calibration point. The colored dots indicate private Sardinian clusters with an average number of SNPs in the range of 35 to 40 in red, 25 to 30 in green, and 7 to 12 in blue. The black dots indicate clusters with an average number of SNPs in the range of 70 to 120. The arrow indicates the position on the tree of the Ötzi, Tuscan, and Corsican samples. The gray box is enlarged in Fig. 2.

family (5), which also coincides with that traditionally deduced from the *Homo-Pongo* divergence (15).
Using our phylogenetic rate of $0.53 \times 10^{-9} \text{ bp}^{-1} \text{ year}^{-1}$, we estimated the time to the most recent common ancestor (MRCA) of all samples, whose average variability is $1002.6 (\pm 21.2)$ SNPs, at ~200,000 years ago. This is older than previously proposed (16) for the Y chromosome but is in agreement with estimates from a de novo mutation rate in an African Y-chromosome lineage (14) and with the revised molecular clock for humans (7) and the TMRCA estimated from analyses of maternally inherited mtDNA (13, 17).
The main non-African super-haplogroup F-R shows an average variation of $534.8 (\pm 28.7)$ SNPs, corresponding to a MRCA of ~110,000 years ago, in agreement with fossil remains of archaic *Homo sapiens* out of Africa (7, 18) though not with mtDNA, whose M and N super-haplogroups coalesce at a younger age (13). The main European subclades show a differentiation predating

the peopling of Sardinia, with an average variation ranging from 70 to 120 SNPs (Table 1), corresponding to a coalescent age between 14,000 and 24,000 years ago, which is compatible with the postglacial peopling of Europe.
However, the inferred phylogenetic rate and dating estimates presented here remain tentative, because the calibration date was deduced from archaeological data, which may be incomplete and typically covers a relatively large temporal interval. In the future, a more precise calibration point might be obtained by sequencing ancient DNAs from prehistoric Sardinian remains dated by radiocarbon methods. Further limitations derive from the scarcity of related samples for rare lineages, coupled with the low-pass sequencing approach we used (8). Low-pass sequencing is expected to detect nearly all common variants (frequency >1%) but to miss rare variants. Missed variants have competing effects on estimates of ancestral coalescent times: When they lead to missed differences among haplotypes that diverged after the founding of Sardinia, they lower

our calibrated estimates of mutation rate and increase coalescent time estimates; when they lead to missed differences among ancestral clades, they lower these time estimates. In fact, despite the overall homogeneity of the length of the branches from the MRCA (Fig. 1), those represented by fewer individuals are generally shorter (8). To estimate the effect of missed variants on the age estimates, we sequenced with deep coverage 7 selected individuals, 4 of them belonging to the I2a1a- δ clade, used for calibration, and 3 to the I2a1a- β , J2b2f and A1b1b2b clades. The deep sequencing of the I2a1a- δ samples yielded an average of $45.7 (\pm 2.2)$ Sardinian-specific SNPs among these haplotypes [versus $37.3 (\pm 7.8)$ in low-coverage data], corresponding to a phylogenetic rate of $0.65 \times 10^{-9} \text{ bp}^{-1} \text{ year}^{-1}$. Overall, this reanalysis suggested a slightly more recent MRCA (~8% lower), still in substantial agreement with the antiquity of the main Y-chromosome haplogroups (8).
Hence, despite current limitations, the calibration used from common haplogroups in over

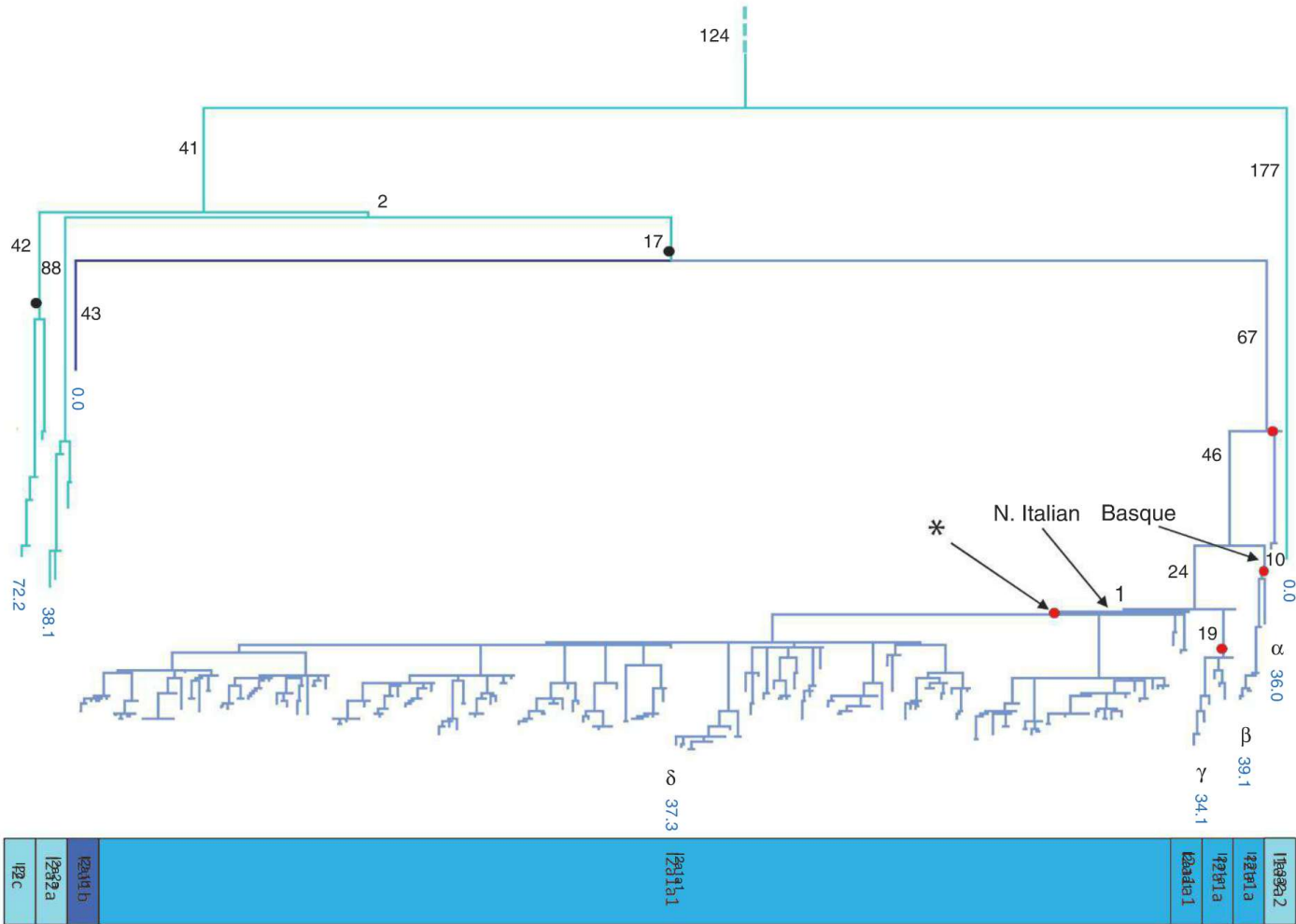


Fig. 2. Phylogenetic tree of the 492 (490 Sardinians and 2 non-Sardinians) Y-chromosome sequences belonging to haplogroup I. The number of polymorphisms for the main branches is shown in black; the average number of SNPs of sub-haplogroups is shown in blue. The sub-haplogroups are named according to

ISOGG nomenclature. The red dots indicate Sardinian private clades, labeled in Greek letters as in Table 1. The black dots indicate clusters with an average number of SNPs in the range of 70 to 120. The arrows indicate the position of the northern Italian and Basque samples on the tree. The asterisk indicates the calibration point.

1000 people from this isolated population, including many island-specific SNPs, permits an estimate of main demographic events during the peopling of Sardinia that is concordant with the archaeological/historical record and ancient DNA analysis (8). The initial expansion of the Sardinian population, used for calibration, is marked by six clades belonging to three different haplogroups, with an average variation of around 35 to 40 SNPs, representing the ancient founder core of modern Sardinians.

Our data further suggest a more intricate scenario of Sardinian demographic history. Specifically, clades of E, R, and G that show Sardinian-specific variability of 25 to 30 SNPs are consistent with further expansion in the Late Neolithic (~5500 to 6000 years ago) (Table 1). Additional variation putatively arrived with groups of individuals carrying other haplogroups (namely the I clades different from I2a1, J, and T). Taken together, the genetic data and demographic expansions are consistent with classical archaeological data indicating that Sardinia reached a considerable population size in prehistoric times; the estimated population during the Nuragic Period (~2500 to 3700 years ago) was >300,000 inhabitants (19). Finally, the rare, mostly African A1b-M13 and E1a-M44 clades could have come to Sardinia in more recent times, up to the historic period correspond-

ing to the Roman and Vandalic dominations, suggested by a private Sardinian variability of 7 to 10 SNPs.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/341/6145/565/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S8
Tables S1 to S3
References (20–37)

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The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic T_{reg} Cell Homeostasis

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Regulatory T cells (T_{regs}) that express the transcription factor Foxp3 are critical for regulating intestinal inflammation. Candidate microbe approaches have identified bacterial species and strain-specific molecules that can affect intestinal immune responses, including species that modulate T_{reg} responses. Because neither all humans nor mice harbor the same bacterial strains, we posited that more prevalent factors exist that regulate the number and function of colonic T_{regs}. We determined that short-chain fatty acids, gut microbiota-derived bacterial fermentation products, regulate the size and function of the colonic T_{reg} pool and protect against colitis in a *Ffar2*-dependent manner in mice. Our study reveals that a class of abundant microbial metabolites underlies adaptive immune microbiota coadaptation and promotes colonic homeostasis and health.

The intestinal immune system has coevolved with the gut microbiota for the maintenance of intestinal health (1). Disruption of this homeostasis leads to intestinal inflammation and disease (2, 3). Colonic regulatory T cells (cT_{regs}) expressing the transcription factor Foxp3 are critical for limiting intestinal inflammation and depend on microbiota-derived signals for proper development and function (4–7). *Bacteroides fragilis* and clostridial species induce T_{reg} responses (6, 7); however, how the gut microbiota

affect cT_{reg} responses across mammalian hosts remains unclear. Although polysaccharide A from *B. fragilis* modulates T_{reg} responses (6), such effects are also likely mediated through more common factor(s) produced by many bacterial genera.

Humans and mice rely on bacteria to break down undigestible dietary components, such as fibers (8). Short-chain fatty acids (SCFAs) are bacterial fermentation products and range in concentration from 50 to 100 mM in the colonic lumen (9). We examined SCFA concentrations in

specific pathogen-free (SPF) mice, gnotobiotic altered Schaedler flora (ASF)-colonized mice, and germ-free (GF) mice and found that GF mice had reduced concentrations of the three most abundant luminal SCFAs—acetic acid, propionic acid, and butyric acid (table S1)—as previously reported (10) (see also supplementary materials and methods). The decrease of these SCFAs in GF mice suggests that SCFAs may contribute to their immune defects, specifically reduced cT_{reg} numbers. We provided SCFAs in the drinking water (150 mM) to GF mice for 3 weeks and found that SCFAs individually or in combination (SCFA mix) increased cT_{reg} frequency and number (Fig. 1A) but did not increase the number or frequency of splenic, mesenteric lymph node (MLN) cells or thymic T_{regs} (fig. S1). These effects coincided with increased luminal SCFAs (table S1). SCFAs increased CD4⁺ T cell frequency and number (fig. S2) but did not alter colonic T helper 1 (T_H1) or T_H17 cell numbers significantly (fig. S3).

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Microbiota-induced cT_{reg} development is associated with increases in de novo generation of inducible T_{reg} s (iT_{reg} s), not T_{reg} s of thymic origin (nT_{reg} s) (7). These populations can be distinguished by expression of Helios, which, in vivo, is restricted to nT_{reg} s (11). We found that Helios⁺ T_{reg} frequency was similar between GF and SCFA-treated GF mice (fig. S4) but was lower in SPF mice. SCFA-treatment increased Helios⁺ T_{reg} numbers in GF mice, indicating that T_{reg} s already present in the colonic lamina propria (cLP) were expanding.

To test if SCFAs could affect cT_{reg} s in a GF setting, we isolated cT_{reg} s from GF mice treated with propionate in vivo for 3 weeks and examined expression of *Foxp3* and *interleukin-10* (*IL-10*), a key cytokine in T_{reg} -mediated suppression. We also isolated cT_{reg} s from GF mice and stimulated them in vitro with propionate. Both treatments increased *Foxp3* and *IL-10* expression significantly (Fig. 1, B and C). In vitro treatment increased *IL-10* production but not that of transforming growth factor- β (TGF β), a T_{reg} -mediated suppression factor, suggesting that SCFAs specifically induce *Foxp3*⁺*IL-10*-producing T_{reg} s (Fig. 1, B and C).

The antibiotic vancomycin, which targets Gram-positive bacteria and disrupts the gut microbiota (12), reduces cT_{reg} s to similar levels as

observed in GF mice (7) (fig. S5). However, when SPF mice were treated with a combination of vancomycin and SCFAs, the reduction in cT_{reg} s was completely restored (fig. S5). Collectively, these results suggest that SCFAs play a role in cT_{reg} homeostasis.

We questioned whether SCFAs would augment cT_{reg} s in SPF mice. SCFA treatment of SPF mice increased *Foxp3*⁺ and *Foxp3*⁺*IL-10*⁺ cT_{reg} frequency and number (Fig. 2, A to C), but not *Foxp3*⁺TGF β ⁺ cT_{reg} s (fig. S6). We did not observe changes with SCFA treatment in small intestinal T_{reg} numbers (fig. S7). Neither colonic T_H17 and T_H1 nor MLN cell and splenic T_{reg} frequency and number from SPF mice were affected by SCFAs (fig. S8 to S11). These results may explain the benefits of dietary fibers and bacteria, such as clostridia and bifidobacteria (13), that can increase colonic luminal SCFA production and modulate inflammation in mice and humans. We measured SCFA production of species belonging to *Clostridium* clusters XI (*Clostridium bifermentans*), XIV (ASF 356 and 492) and XVII (*C. ramosum*) and the bacteroides species *B. fragilis*, as *Clostridium* cluster XIV members and *B. fragilis* affect cT_{reg} s (6, 7). ASF 356 and 492 and *C. ramosum* generated more propionate [14 to 62 versus 0.05 to 1.1 $\mu\text{mol}/10^5$

colony-forming units (CFU)] and acetate (118 to 220 versus 0.1 to 2 $\mu\text{mol}/10^5$ CFU) compared with the other strains (table S1).

Colonic regulatory T cells regulate intestinal homeostasis and control inflammation by limiting proliferation of effector $CD4^+$ T cells (T_{eff}). Addition of SCFAs to cT_{reg} and T_{eff} cocultures increased cT_{reg} suppressive capacity (Fig. 2D and fig. S12). In SPF mice, SCFAs are taken up by colonic epithelial cells but also diffuse through the epithelium into the lamina propria, where they can mediate their effects directly (9, 14). To determine if SCFAs directly affect cT_{reg} s, we isolated cT_{reg} s from SCFA-treated SPF mice. In vivo treatment increased cT_{reg} *Foxp3* and *IL-10* expression (Fig. 2E). We also isolated cT_{reg} s from SPF mice and incubated them with SCFAs in vitro. *Foxp3* expression, as well as *IL-10* expression and protein production, increased (fig. S13), whereas TGF β levels remained unchanged (fig. S14). As enhanced suppressive activity (Fig. 2D) could be attributed not only to higher *IL-10* levels per cT_{reg} but also to increased cT_{reg} proliferation, we examined cT_{reg} proliferation. cT_{reg} s exhibited enhanced proliferation when cultured in the presence of propionate (fig. S15).

In addition, we analyzed the expression patterns of T_{reg} trafficking molecules. Although

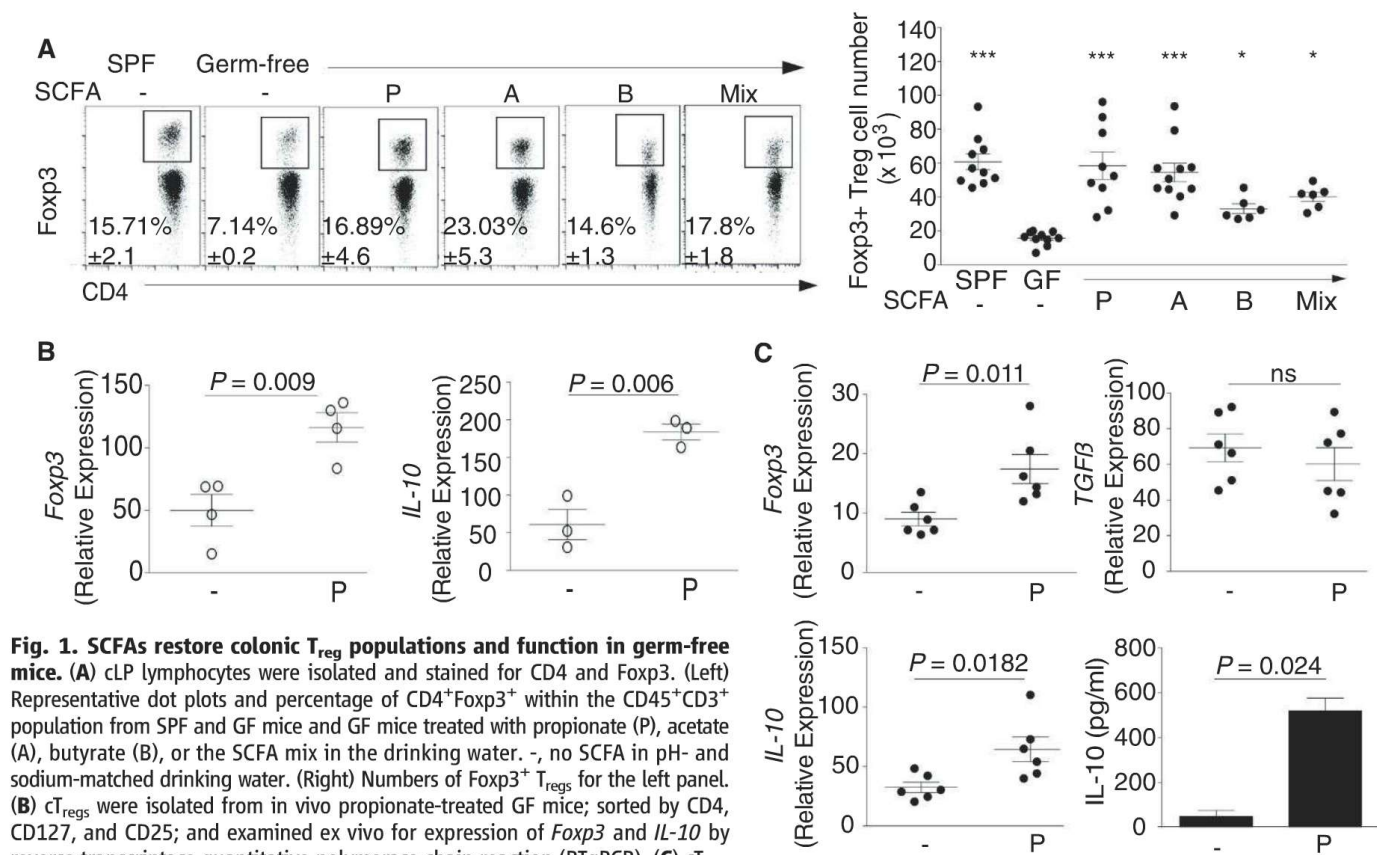


Fig. 1. SCFAs restore colonic T_{reg} populations and function in germ-free mice.

(A) cLP lymphocytes were isolated and stained for CD4 and *Foxp3*. (Left) Representative dot plots and percentage of $CD4^+$ *Foxp3*⁺ within the $CD45^+$ $CD3^+$ population from SPF and GF mice and GF mice treated with propionate (P), acetate (A), butyrate (B), or the SCFA mix in the drinking water. -, no SCFA in pH- and sodium-matched drinking water. (Right) Numbers of *Foxp3*⁺ T_{reg} s for the left panel. (B) cT_{reg} s were isolated from in vivo propionate-treated GF mice; sorted by CD4, CD127, and CD25; and examined ex vivo for expression of *Foxp3* and *IL-10* by reverse transcriptase quantitative polymerase chain reaction (RTqPCR). (C) cT_{reg} s were isolated from GF mice and purified as in (B); cultured for 24 hours in the presence or absence of 0.1 mM propionate; and examined for expression of *Foxp3*, TGF β , and *IL-10* by RTqPCR and *IL-10* protein production by enzyme-linked immunosorbent assay. In (A), symbols represent data from individual mice. Horizontal lines show the mean; error bars denote the SD. In (B) and (C), each symbol or bar represents pooled cT_{reg} s from three to five mice. All data shown are representative of at least three independent experiments. (A) Kruskal-Wallis test with a Dunn's post hoc test: *** $P < 0.001$; * $P < 0.05$. (B and C) Mann-Whitney U test. ns, not significant.

levels of the chemokine receptor *CCR9* or $\alpha_4\beta_7$ integrin were not altered in propionate-treated GF and SPF mice, levels of the cT_{reg} homing receptor, *G protein-coupled receptor 15* (*GPCR15*) (15), did increase (fig. S16). Taken together, these data indicate that SCFAs may have a beneficial effect in SPF mice through their ability to increase Foxp3⁺IL-10-producing cT_{reg}s and cT_{reg} proliferative capacity, as well as to alter cT_{reg} *GPCR15* expression.

Considering that SCFAs can influence cT_{reg}s directly, we asked if this was a receptor-mediated process. GPCR43 (*Ffar2* is the gene that encodes GPCR43) binds SCFAs and, through its expression on innate immune cells, mediates resolution of inflammatory responses (3, 16). We found that cT_{reg}s had significantly higher levels of *Ffar2* than T_{reg}s isolated from the spleen or MLN (Fig. 3, A and B), and *Ffar2* levels were largely dependent on microbiota-derived signals. As a reference, we compared cT_{reg} *Ffar2* expression levels to colonic myeloid (CD11b⁺) cells, which are known to express *Ffar2* (3), and found that, on average, CD11b⁺ cells expressed 1.6-fold more *Ffar2* than cT_{reg}s (fig. S17).

To determine if *Ffar2* contributes to cT_{reg} homeostasis, we treated *Ffar2*^{-/-} mice and *Ffar2*^{+/-} littermates with propionate, which has the highest affinity for *Ffar2* (17), and measured cT_{reg} numbers. Propionate enhanced cT_{reg} frequency and number in *Ffar2*^{+/-}, but not *Ffar2*^{-/-} mice (Fig. 3C). SCFA-mediated, enhanced cT_{reg} suppressive capacity was also dependent on *Ffar2* (Fig. 3D). In addition, propionate enhanced *Foxp3* and *IL-10* expression and IL-10 protein levels in *Ffar2*^{+/-} cT_{reg}s, but not in *Ffar2*^{-/-} cT_{reg}s (fig. S18). Furthermore, we examined whether propionate could restore cT_{reg} populations and numbers in the setting of vancomycin treatment and found that *Ffar2* was necessary (fig. S19).

One mechanism by which SCFAs mediate their effects is histone deacetylase (HDAC) inhibition (18). The HDAC inhibitor trichostatin A increases T_{reg} gene expression and suppressive capacity (19), and HDAC6 and -9 down-regulate nT_{reg} function (20). Given that SCFAs promote cT_{reg} homeostasis in our studies, we hypothesized that SCFAs mediate their effect through HDAC inhibition. We treated *Ffar2*^{+/-} and *Ffar2*^{-/-} mice with propionate and measured HDAC expression.

Propionate treatment of *Ffar2*^{+/-} mice reduced cT_{reg} *HDAC6* and *HDAC9* (class IIb and IIa, respectively) expression but did not reduce *HDAC1* and *HDAC2* (class I) or *HDAC7* (class IIa) expression (Fig. 3E). Western blot analysis demonstrated that propionate treatment of cT_{reg}s enhanced histone acetylation, which was dependent on *Ffar2* expression (Fig. 3F). These results suggest that SCFAs via *Ffar2* may affect cT_{reg}s through HDAC inhibition.

To further evaluate the effects of SCFAs on cT_{reg}s, we used the T cell-transfer model of colitis (21). In this model, lymphopenic mice (e.g., *Rag2*^{-/-}) are injected with either naïve T cells, which results in severe colitis, or naïve T cells in combination with T_{reg}s, which reduces colitis severity. Propionate or SCFA mix-treated mice injected with naïve T cells and T_{reg}s had less severe weight loss, reduced disease score, and lower levels of colonic proinflammatory cytokines than mice that received water alone (Fig. 4A and fig. S20). SCFA mix or propionate did not affect colitis severity in mice that received only naïve T cells (fig. S20). The frequency and number of cLP Foxp3⁺ T_{reg}s in mice receiving

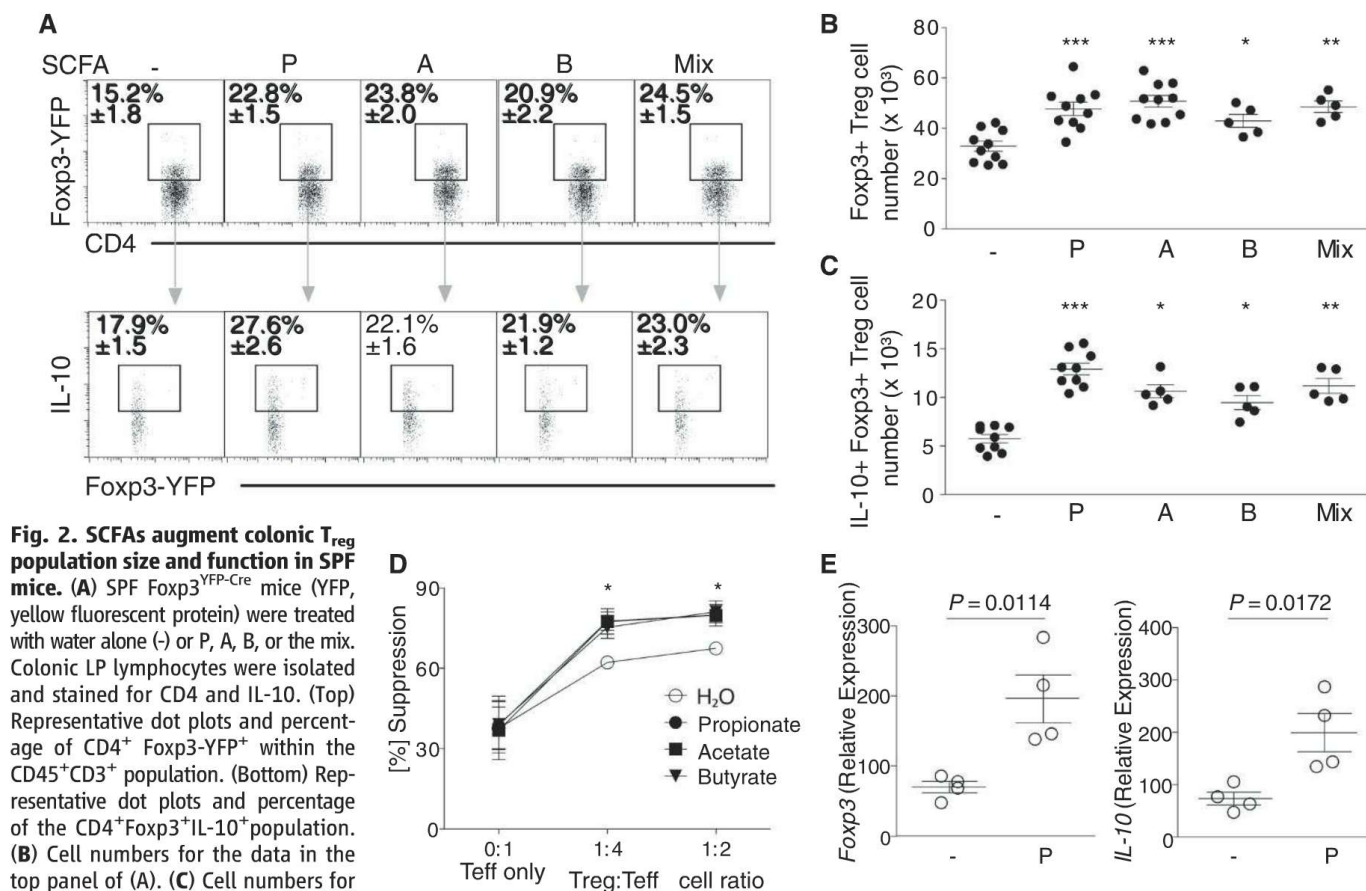
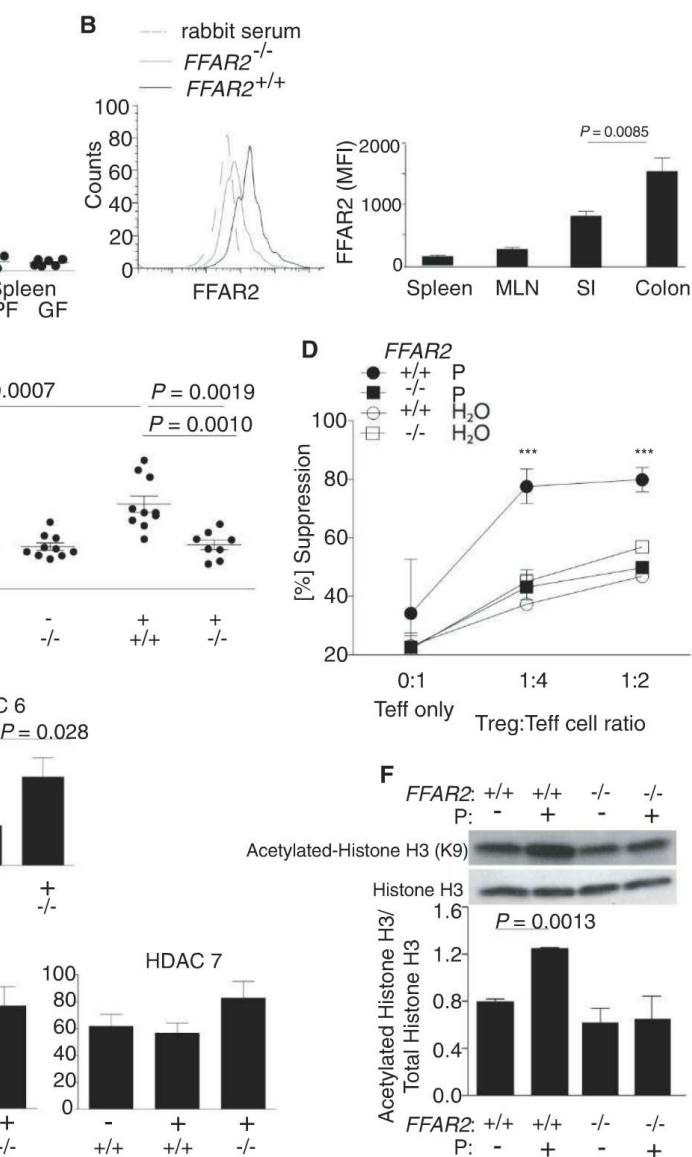


Fig. 2. SCFAs augment colonic T_{reg} population size and function in SPF mice. (A) SPF Foxp3^{YFP-Cre} mice (YFP, yellow fluorescent protein) were treated with water alone (-) or P, A, B, or the mix. Colonic LP lymphocytes were isolated and stained for CD4 and IL-10. (Top) Representative dot plots and percentage of CD4⁺ Foxp3-YFP⁺ within the CD45⁺CD3⁺ population. (Bottom) Representative dot plots and percentage of the CD4⁺Foxp3⁺IL-10⁺ population. (B) Cell numbers for the data in the top panel of (A). (C) Cell numbers for the data in the bottom panel of (A). Symbols in (B) and (C) represent data from individual mice and four independent experiments. (D) cT_{reg}s were cocultured with splenic T_{eff} and P, A, B, or media (sodium- and pH-matched) for 96 hours. Percent suppression is shown on the y axis; the x axis denotes T_{reg}:T_{eff} ratios. Symbols represent mean values; error bars denote SD for four independent experiments. **P* < 0.01. (E) cT_{reg}s were isolated from the LP of in vivo propionate-treated SPF

Foxp3^{YFP-Cre} mice, sorted for CD4 and YFP, and examined for ex vivo expression of *Foxp3* and *IL-10*. Each symbol represents pooled cT_{reg}s from three to five mice, horizontal lines show the mean, and error bars denote SD. Four independent experiments were performed. (B, C, and D) Kruskal-Wallis test with a Dunn's post hoc test: ****P* < 0.0001; ***P* < 0.01; **P* < 0.05. (E) Mann-Whitney U test: *P* values are shown where significant.

Fig. 3. Ffar2 mediates SCFA effects on cT_{regs}.

(A) T_{regs} were isolated from the colon, small intestine (SI), spleen, and MLN of SPF and GF BALB/c mice and purified as in Fig. 1B, and *Ffar2* expression was examined by qPCR. Each symbol represents data from three to five individual mice, horizontal lines show the mean, and error bars indicate SD. Data reflect three to seven independent experiments. (B) Lymphocytes were isolated from the colon, small intestine, spleen, and MLN of SPF *Ffar2*^{-/-} and littermate *Ffar2*^{+/+} mice. Cells were stained for CD4, Foxp3, and *Ffar2*. (Left) A representative flow cytometry histogram comparing colonic *Ffar2* expression in *Ffar2*^{-/-} versus littermate *Ffar2*^{+/+} mice. (Right) mean fluorescence intensity (MFI) for *Ffar2* for T_{regs} from the indicated sites. Bars show the mean, error bars denote SD, and data reflect four independent experiments. (C) Colonic LP lymphocytes were isolated from *Ffar2*^{-/-} and littermate *Ffar2*^{+/+} mice exposed to propionate (P) or water alone and stained for CD4 and Foxp3. (Left) Representative dot plots with percentage of CD4⁺Foxp3⁺ within the CD45⁺CD3⁺ population. (Right) Foxp3⁺ T_{reg} number for the left panel. Each symbol represents data from individual mice, horizontal lines show the mean, and error bars indicate SD. (D) *Ffar2*^{-/-} and littermate *Ffar2*^{+/+} cT_{regs} were cocultured with splenic T_{eff} cells in media with or without propionate for 96 hours. Percent suppression, y axis; T_{reg}:T_{eff} ratios, x axis. Symbols represent the mean of three independent experiments; error bars show the SD. (E) cT_{regs} were isolated from the LP of *Ffar2*^{-/-} and littermate *Ffar2*^{+/+} mice; purified as in Fig. 1B; cultured in the presence of 0.1 mM propionate or media (pH- and sodium-matched) for 24 hours; and examined for expression of *HDAC1*, *-2*, *-6*, *-7*, and *-9* by RTqPCR. Bars show the mean and error bars the SD of three independent experiments. (F) Whole-cell extracts were



generated from cT_{regs} isolated from the LP of *Ffar2*^{-/-} and littermate *Ffar2*^{+/+} mice, purified as in Fig. 1B, and cultured in the presence of 0.1 mM propionate or media (pH- and sodium-matched) for 24 hours. Samples were analyzed by Western blotting for histone acetylation by examining levels of acetylated histone (H3K9); total histone levels were used as a loading control. The Western blot shown is representative of two independent experiments with cT_{regs} cell lysates pooled from 10 to 12 mice per group. A bar graph of densitometry ratios of acetylated histone H3:total histone H3 is shown. Bars represent the mean; error bars denote SD. (A and D) Kruskal-Wallis test with a Dunn's post hoc test: *** $P < 0.001$. (C and E) Mann-Whitney U test. (B and F) Student's *t* test.

propionate and SCFA mix increased (Fig. 4, B and C). SCFAs, however, did not result in conversion of naïve T cells to cT_{regs} (Fig. 4, B and C). To evaluate whether these effects were cT_{reg}-intrinsic and dependent on *Ffar2*, we performed the T cell-transfer colitis model using *Rag2*^{-/-} recipients, wild-type naïve T cells, and *Ffar2*^{+/+} or *Ffar2*^{-/-} T_{regs} with or without propionate in the drinking water (Fig. 4D and fig. S21). The effect of propionate on intestinal inflamma-

tion was dependent on *Ffar2* expression in T_{regs}, as indicated by the colitis scores (Fig. 4D). T_{reg} cell populations and numbers further substantiated our finding that the propionate effects on cT_{regs} were dependent on *Ffar2* (Fig. 4, E and F).

Gut microbiota-host immune misadaptation has been implicated in the rising incidence of inflammatory bowel disease, other inflammatory diseases, and obesity (22). The Western dietary

pattern, specifically reduced ingestion of plant-based fibers, may be a critical factor that links the gut microbiome to disease (23). Although the gut microbiota composition is divergent across individuals, functional gene profiles are quite similar (24, 25), and alterations to common gut microbial metabolic pathways may affect the production of symbiotic factors, such as SCFAs, which regulate intestinal adaptive immune responses and promote health.

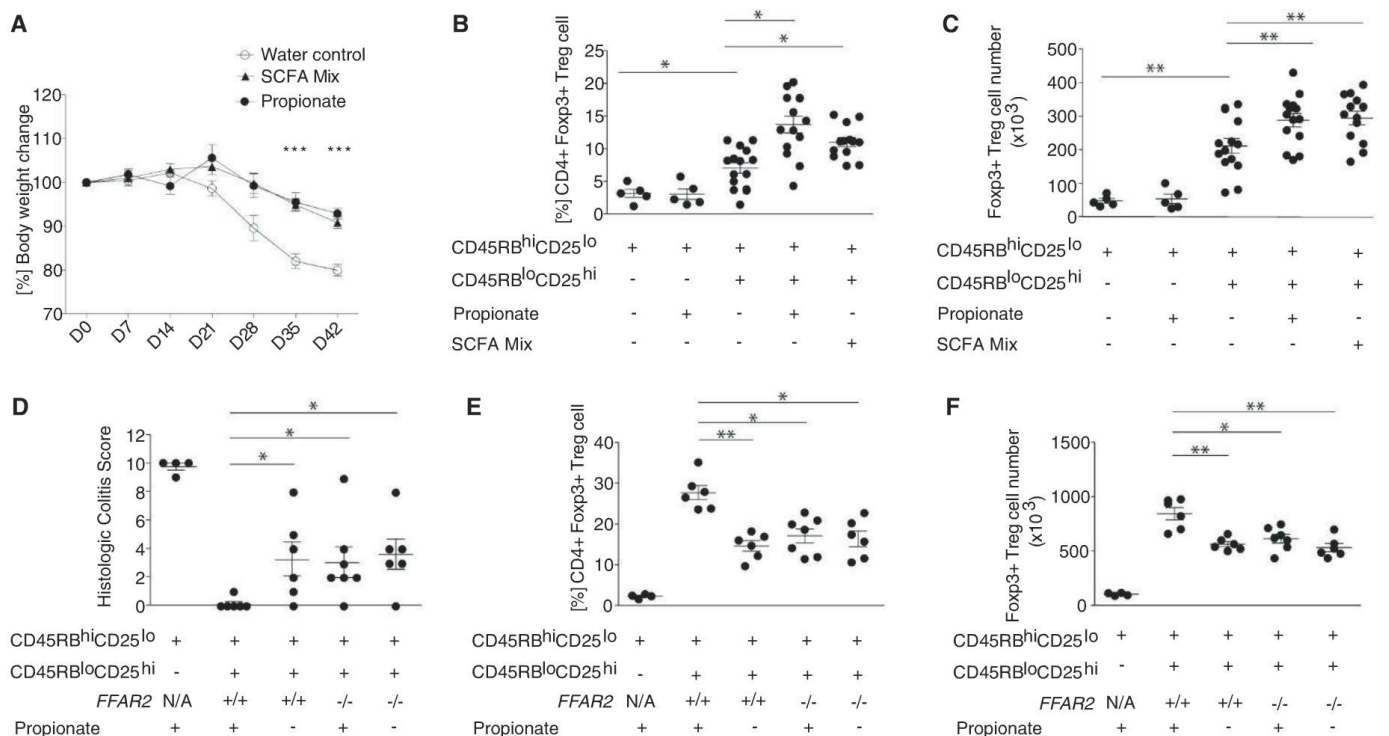


Fig. 4. SCFA exposure ameliorates T cell-transfer colitis in a T_{reg}-intrinsic, *Ffar2*-dependent manner. BALB/c *Rag2*^{-/-} mice were injected with CD4⁺CD45RB^{hi}CD25^{lo} naïve T cells alone or in combination with T_{regs}. After injection, mice received propionate, SCFA mix, or pH- and sodium-matched drinking water. (A) Weekly percentage of body weight change is shown across the experimental groups from experimental days 0 to 42 (D0 to D42). Symbols show the mean; error bars indicate SD. Data reflect three independent experiments. Colonic LP lymphocytes were isolated and stained for CD4 and Foxp3, and (B) percentage and (C) number of CD4⁺Foxp3⁺ within the CD45⁺CD3⁺ population are shown. Symbols represent data from individual mice, horizontal lines show the mean, and error bars denote SD.

(D to F) C57BL/6 *Rag2*^{-/-} mice were injected with CD4⁺CD45RB^{hi}CD25^{lo} naïve T cells alone or in combination with *Ffar2*^{+/-} or *Ffar2*^{-/-} T_{regs}. After injection, mice received propionate or pH- and sodium-matched drinking water. (D) Histologic colitis score is shown along the y axis, the treatment group and experimental conditions are shown along the x axis. Colonic LP lymphocytes were isolated, and (E) percentage and (F) number of CD4⁺Foxp3⁺ within the CD45⁺CD3⁺ population are shown. Symbols represent data from individual mice. Horizontal lines show the mean, and error bars denote the SD. Data in (D) to (F) are from two independent experiments. (A to F) Kruskal-Wallis test with a Dunn's post hoc test: ****P* < 0.001; ***P* < 0.01; **P* < 0.05. N/A, not applicable.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1241165/DC1
Materials and Methods
Figs. S1 to S24
Table S1
References (26–28)

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The Schmid College is a leader in applying interdisciplinary problem-solving to global issues through teaching, research and discovery, with strengths in computational sciences and quantum physics. The College is home to the School of Computational Sciences, solving real-world problems using data mining, signal and image processing, mathematical modeling and simulation, and predictive analytics; and to the School of Earth and Environmental Sciences, educating tomorrow's problem solvers through interdisciplinary research and programs in biological sciences, chemistry, environmental science and policy, food science, hazards and global environmental change. The Schmid College employs an internationally renowned faculty comprised of a National Medal of Science Awardee, two National Science Foundation Career Award Recipients, and a Dreyfus Teacher-Scholar Awardee, among others.

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Applicants and those seeking further information should contact **Betty Hasler or Kim M. Morrisson at Diversified Search at Chapman_Schmid@divsearch.com**. Materials should be submitted electronically and should include a cover letter, resume/curriculum vitae, and the names of five references. All inquiries will remain confidential.

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The Division of Life Sciences in the UCLA College of Letters and Sciences announces the continuance of its special initiative to recruit excellent research scientists with a history and commitment to the mentorship of students from underrepresented and underserved populations. Candidates should have outstanding records of scholarly publications, research support, and teaching and be eligible for Academic Senate appointment in one of the following departments of Life Sciences (www.lifesciences.ucla.edu): Ecology and Evolutionary Biology; Integrative Biology and Physiology; Microbiology, Immunology, and Molecular Genetics; and the UCLA Institute for Society and Genetics. The successful candidate will be expected to continue his/her active research program, mentor undergraduates, and participate in campus-wide and departmental programs that provide research and professional development opportunities for our diverse student body, including MARC, (Minority Access to Research Careers), PEERS (Program for Excellence in Education & Research in Sciences), and the Biomedical Research Minor. Teaching loads and assignments will take into account mentorship activities associated with the position. Faculty appointment will be made at a professorial rank commensurate with current academic standing and achievement. UCLA offers competitive salaries, research set-up funds, and recruitment allowances.

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Complete applications submitted by October 31, 2013 will receive full consideration.



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Research Position at ICYS, NIMS, Japan



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In the ICYS, we offer a special environment that enables young scientists to work independently based on their own idea and initiatives. All management and scientific discussions will be conducted in English. An annual salary between 5.03 and 5.35 million yen (level of 2012) will be offered depending on qualification and experience. The basic contract term is two years and may be renewed to one additional year depending on the person's performance. A research grant of 2 million yen per year will be supplied to the ICYS researcher.

All applicants must have obtained a PhD degree within the last ten years. Applicants should submit an application form, which can be downloaded from our web site, together with a resume (CV) and a list of publications. A research proposal on an interdisciplinary or integrated area related to the materials science should also be submitted. The application letter should reach the following address via e-mail or air mail by **September 30, 2013**. Visit our website for more details (<http://www.nims.go.jp/icys/>).

**ICYS Administrative Office,
National Institute for Materials Science
Sengen 1-2-1, Tsukuba, Ibaraki 305-0047, Japan
e-mail: icys-recruit@nims.go.jp**



Cleveland Clinic Faculty Positions in Cancer Research Department of Cancer Biology Lerner Research Institute (LRI)

The Department of Cancer Biology is seeking multiple cancer researchers at all levels (Assistant/Associate/Full Professor) with a focus in hematological malignancies/multiple myeloma, kidney cancer, or prostate cancer with strong translational interests. The positions provide an exceptional opportunity to translate basic discoveries to the clinic through collaborative interactions with outstanding clinical programs.

The Department of Cancer Biology currently has 9 primary Faculty actively involved in basic and translational research programs investigating cancers of the brain, colon/rectum, kidney, and prostate, as well as hematologic malignancies. Research foci include cell signaling, DNA damage and repair, tumor suppressor genes, new targets, histone modification, cell cycle control, developmental therapeutics, drug resistance, invasion, metastasis, angiogenesis, tumor microenvironment, and the innate defense against viruses and cancer. The LRI offers over twenty Clinic-subsidized Core services including the Animal Tumor Core, Mass Spectrometry, Imaging and Confocal Microscopy, Genomics, and the Small Molecule Screening Core, allowing access to the latest state-of-the-art equipment.

To be considered, applicants must have M.D., M.D./Ph.D., or Ph.D. degrees and must have ongoing grant support and an accomplished research program in above-mentioned cancers. The successful applicants will be supported by generous start-up funds and joint appointment in Cleveland Clinic's Taussig Cancer Institute (part of the NCI designated Case Comprehensive Cancer Center) and Lerner College of Medicine.

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For further information see: <http://www.lerner.ccf.org/cancerbio/>.

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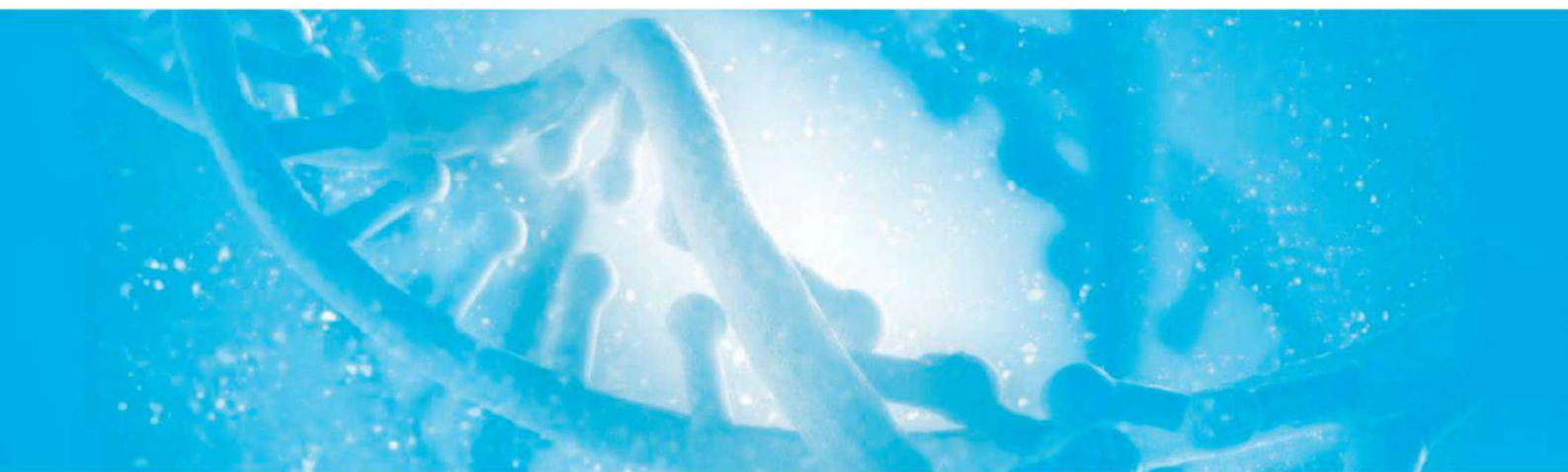
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Ph.D. or equivalent degree (post-doctoral experience is preferred) in ruminant nutrition preferably with emphasis in rumen microbiology or related field with interest in enhancing efficiency of food production, nutrient retention, and qualities of human food products and the effect of the gut microbiome population and/or function in this system. The research is expected to have application to production animals. Evidence of research excellence is expected. The candidate should have the ability to develop and instruct undergraduate and graduate courses, and to design and conduct extramurally-funded research relevant to sustainable animal production and health.

Application materials must be submitted via the following website: <https://recruit.ucdavis.edu/apply/JPF00104>. The position will remain open until filled. To ensure consideration, applications should be received by **October 1, 2013**.

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ANNOUNCEMENTS

futureearth research for global sustainability

Call for expressions of interest to host the permanent secretariat for Future Earth

The Science and Technology Alliance for Global Sustainability is launching an open competitive process for the selection of a permanent secretariat for Future Earth, a 10-year international research programme dedicated to generating new knowledge for challenges posed by global environmental change and transitions towards global sustainability. The call is being managed by the International Council for Science (ICSU).

The successful bid will host a permanent secretariat – which should comprise a headquarters and regional nodes – for the 10-year research programme. The secretariat will coordinate research activities, handle the day-to-day management of Future Earth, and liaise with key stakeholders.

Interested parties (whether individual organisations or consortia of organisations) are invited at this stage to express their interest by reviewing the briefing document and completing a template (both available at <http://bit.ly/futureearthsecretariat>). Following this first step (expression of interest), a bidders' conference (step 2) will be organised to discuss the requirements further and enable bidders to meet with other potential bidders so that they can self-organize for the call for full proposals (step 3) where bidders are expected to submit full proposals (comprising a headquarters and regional nodes, with secured funding). The Future Earth secretariat is expected to be appointed in mid-2014 and be fully operational by the end of 2014.

All expressions of interest should be submitted not later than

23 September 2013

For more information on Future Earth and the Alliance, see <http://www.futureearth.info/> and <http://www.icsu.org/future-earth/>



Roche Translational & Clinical Research Center (TCRC)

Request for Proposals for Young Investigator Award

Roche is pleased to announce that we are accepting applications for the 2013 TCRC Young Investigators Award. The awards are intended to provide support for the earlier stages of scientific research career development, so they are to be awarded to the employing institutions of promising emerging researchers. Proposals will be evaluated based on scientific merit and innovation of the projects proposed to be conducted using the awards. Four awards will be presented on Oct 1, 2013 during the scientific symposium inaugurating Roche's Translational and Clinical Research Center in New York City. Researchers performing investigations (clinical, in vitro/in vivo or informatics) focused on disease understanding of the following disease areas are encouraged to apply:

Cardiovascular & Metabolism – Diabetes and related complications

Neuroscience – Spinal Muscular Atrophy

Virology – Influenza

Oncology – Treatment and diagnosis of Acute Myeloid Leukemia (AML).

Program Overview and Eligibility: Eligibility is limited to researchers performing investigations (clinical, in vitro/in vivo or informatics) in the foregoing disease areas who have completed their graduate degrees (MD and or Ph.D) within the past 5 years. Four awards of Fifty Thousand (\$50,000 USD) Dollars will be granted to the employing institutions of each selected researcher in North America to be used , towards extending fellowship training, funding for new project, or as supplemental funding for ongoing projects.

Application Process:

All applications will be reviewed and selected by an internal TCRC committee comprised of Discovery & Translational Medicine experts. All awards must comply with TCRC policies and processes. TCRC, in its sole discretion, reserves the right to modify the terms of any proposal, eligibility, program, award, policy and process at any time for any reason.

Submission deadline: August 30th, 2013

Email your submissions to: tcrc.humanresources@roche.com indicating your therapeutic area of interest. Please attach the following:

- One page summary of research project
- Curriculum Vitae

Timelines:

- August 30th – Submissions deadline
- September 16th – Notification to recipients
- October 1st – Award presentation
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DR. SHIRLEY MALCOM



To Dr. Shirley Malcom, born and raised in the segregated South more than 65 years ago, a career based on her studies in science seemed even less likely than the launch of the Soviet's Sputnik. But with Sputnik's success, the Space Race officially started and, in an instant, brought a laser-like focus to science education and ways to deliver a proper response. Not long after, Dr. Malcom entered the picture.

Although black schools at the time received fewer dollars per student and did not have sufficient resources to maintain their labs at a level equivalent to the white schools, Dr. Malcom found her way to the University of Washington where she succeeded in obtaining a B.S. in spite of the difficulties of being an African American woman in the field of science. From there she went on to earn a Ph.D. in ecology from Penn State and held a faculty position at the University of North Carolina, Wilmington.

Dr. Malcom has served at the AAAS in multiple capacities, and is presently Head of the Directorate for Education and Human Resources Programs. Nominated by President Clinton to the National Science Board, she also held a position on his Committee of Advisors on Science and Technology. She is currently a member of the Caltech Board of Trustees, a Regent of Morgan State University, and co-chair of the Gender Advisory Board of the UN Commission on Science and Technology for Development. She has held numerous other positions of distinction and is the principal author of *The Double Bind: The Price of Being a Minority Woman in Science*.

Of her active career in science, Dr. Malcom says, "I guess I have become a poster child for taking one's science background and using that in many other ways: we ask questions; we try to understand what we find; we consider what evidence we would need to confirm or refute hypotheses. And that happens in whatever setting one finds oneself."

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Faculty Positions Available in Beihang University, China

Established in 1952, located in Haidian District, Beijing, Beihang University is one of the top research-oriented universities in China, focusing on fundamental cutting edge research and high-level education, covering such diverse fields as science, engineering, technology, humanities, economics, management and law. One of the first universities funded by China's "211" and "985" programs, it has seven national key laboratories and twenty-five provincial and ministerial key laboratories. At present, the university has a total area of two million square meters, and over 3800 faculty and staff.

Beihang University is on a clear path to become a world-class university in many engineering and science disciplines. As part of Beihang's further pursuit for excellence in research and education, we have expanded our global search for the best research talent to join our International Research Institute for Multidisciplinary Science (IRIMS). Five independent international research centers (IRC) were established recently under the name of IRIMS. As the core part of IRIMS, IRCs are devoted to establish a world-class, advanced and multidisciplinary research platform.

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- Position offered by Beihang University's Zhuoyue Program of Professors
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Interested individuals should send curriculum vitae by email to rscreb@buaa.edu.cn, with "Faculty Application from *Science*" in the title. For more information, please visit the university's Human Resource Department website <http://rsc.buaa.edu.cn/>, or contact us by email rscreb@buaa.edu.cn or by telephone 86-010-82317779.

Core-funded Junior Faculty Positions

• Job Reference number: PI/13/01-2

The Paterson Institute for Cancer Research (www.paterson.man.ac.uk) is seeking several tenure-track Junior Group Leaders to join a vibrant programme of basic, translational and clinical research. The Paterson Institute for Cancer Research in Manchester (UK) is a centre for excellence in cancer research. We occupy state-of-the-art laboratories and provide exceptional core facilities, including next generation sequencing, mass-spectrometry, advanced imaging, bioinformatics, histology, and flow cytometry. We are core-funded by Cancer Research UK (www.cancerresearchuk.org), the world's largest cancer organisation, are an Institute of The University of Manchester (www.manchester.ac.uk), and are adjacent to The Christie NHS Foundation Trust (www.christie.nhs.uk), one of the largest cancer treatment centres in Europe. These factors combine to provide an exceptional environment in which to pursue basic, translational and clinical research programmes in a dynamic city surrounded by beautiful countryside and with excellent national and international transport links.

We seek outstanding individuals who excel in their research areas to establish vigorous and ambitious independent research programmes. We welcome applications from highly motivated and innovative individuals who are keen to communicate well-formulated research proposals. We are particularly eager to recruit to our priority areas, which currently include:

- Lung cancer • Melanoma immunology • Pancreatic cancer
- Gynaecological cancers • Molecular pathology

The positions are equivalent to Lecturer or Senior Lecturer and the successful applicants will have a PhD in a relevant area of cancer biology, appropriate post-doctoral training, and a proven track record demonstrated

through high-impact publications. They will manage a group of research scientists and students and will be responsible for the professional development of their group members. Junior Group Leaders will be considered for promotion to Senior Group Leader posts at 6 years.

The generous package includes:

- Six years of core-funded support
- Competitive personal salary
- Up to three additional core-funded posts
- Up to two core-funded PhD students or clinical fellows
- Generous running expenses and start-up package for equipment
- High quality laboratory space
- Scope for group expansion through additional external funding
- Excellent core facilities boosted by £8.7m HEFCE investment (2012)

Informal enquiries can be addressed to the Director, Professor Richard Marais, email: rmarais@picr.man.ac.uk

To apply for these positions please send a CV, the names of three referees a short summary of past research and a short research proposal (where possible in a single Adobe PDF document). Also include the CV cover sheet and Equal Opportunities form available at www.paterson.man.ac.uk/ Jobs/. Please submit your application to the HR Department (Email: jobs@picr.man.ac.uk; Tel: +44 (0)161 446 3231).

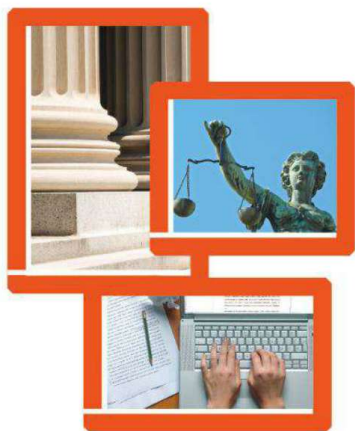
Closing date: 13 September 2013.



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www.paterson.man.ac.uk





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POSITIONS OPEN

DEPARTMENT OF CHEMISTRY

Iowa State University

Experimental Biophysical Chemistry Search

Iowa State University (ISU) Department of Chemistry, located in Ames, Iowa invites applications for a tenure-track position in experimental biophysical chemistry. We are interested in scientists with outstanding accomplishments, a demonstrated potential for imaginative research and an enthusiasm for teaching physical and/or analytical chemistry. Candidates who develop state-of-the-art spectroscopic and/or imaging techniques and instrumentation, and apply these methods to study fundamental biochemical phenomena are especially encouraged to apply. Applicants must have a Ph.D. in chemistry or related discipline. ISU offers a collaborative research environment, a campus-wide initiative on structural biology research, and competitive startup packages. Review of applications will begin on September 25, 2013. Please apply online at [website: https://www.iastatejobs.com](https://www.iastatejobs.com) vacancy #130587.

ISU is an Equal Opportunity/Affirmative Action Employer. Women and members of minorities are particularly encouraged to apply.

FACULTY POSITION in Physiology/Vascular Biology

The University of South Alabama, College of Medicine, seeks applications for a tenure-earning position at the rank of **ASSISTANT PROFESSOR** in the Department of Physiology and the Program of Vascular Biology. Investigators having expertise in hypertension, inflammation, and/or signal transduction are encouraged to apply. The Graduate Program in Basic Medical Sciences consists of over 40 students working toward the Doctorate degree, and applicants will participate in medical and graduate student teaching. Interested scientists should submit curriculum vitae and a letter stating research interests and goals to: **Search Committee, Department of Physiology, College of Medicine, University of South Alabama, 5851 USA Dr. N., MSB Rm. 3074, Mobile, AL 36688.** Applications will be reviewed until the position is filled. *The University of South Alabama is an Equal Opportunity/Equal Access Employer.*

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Contact the Admissions Office, telephone: 800-824-5526 at The New England College of Optometry, 424 Beacon Street, Boston, MA 02115. Additional information, [website: http://www.neco.edu](http://www.neco.edu); e-mail: admissions@neco.edu.

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POSITIONS OPEN

FACULTY POSITION

Pharmaceutics/Pharmaceutical Biotechnology

The Department of Pharmaceutical Sciences at North Dakota State University (NDSU) invites applications for a tenure-track position at the rank of **ASSISTANT/ASSOCIATE PROFESSOR**. The appointment is expected to begin on or after January 1, 2014. Applicants should possess a Ph.D. degree in pharmaceutics, nanotechnology, pharmaceutical biotechnology, biomedical engineering, or suitably related fields, have at least two years of postdoctoral experience with a strong record of scholarship, and possess good interpersonal and communication skills. Preference will be given to those applicants with expertise in novel drug delivery to deliver biotechnologically derived molecules using nanotechnology, to individuals whose research interests complement other departmental strengths, and to individuals with a degree in pharmacy. Successful candidates will be expected to teach pharmaceutical biotechnology and basic pharmaceutics in the Doctor of Pharmacy program, teach and mentor MS/Ph.D. graduate students, and develop extramurally funded research programs.

A highly competitive salary and a startup package commensurate with qualifications and experience are available. The Department of Pharmaceutical Sciences has the mission of teaching pharmacists how basic science is applied to Pharmacy. In addition to teaching professional (Pharm.D.) students, the department has MS, Ph.D. and PharmD/Ph.D. programs and participates in multidisciplinary Ph.D. program in Cellular and Molecular Biology. Currently, there are thirteen faculty members, and about 30 Ph.D. students and postdoctoral Fellows/Research Associates in the department. All of the department faculty have active research program and have grants from NIH, NSF, American Heart Association, and pharmaceutical industries. The department participates in NIH COBRE grant with the Department of Chemistry. Fargo is a rapidly growing city with renowned organizations such as Sanford Health, Microsoft Corp., and Deere & Company (John Deere). Additional information concerning the Department, the University, and the city of Fargo can be obtained at [website: http://www.ndsu.edu/pharmsci/](http://www.ndsu.edu/pharmsci/).

Application deadline is September 30, 2013, or thereafter until the position is filled. The application portfolio containing the curriculum vitae, statement of teaching philosophy, description of research interests and future plans, and three letters of reference must be submitted electronically at [website: http://jobs.ndsu.edu/postings/3792](http://jobs.ndsu.edu/postings/3792). For more information, please contact: **Dr. Bin Guo** (e-mail: bin.guo@ndsu.edu), North Dakota State University College of Pharmacy, Nursing and Allied Sciences, Fargo, ND 58105.

NDSU is an Equal Opportunity Institution. Women and traditionally underrepresented groups are encouraged to apply.

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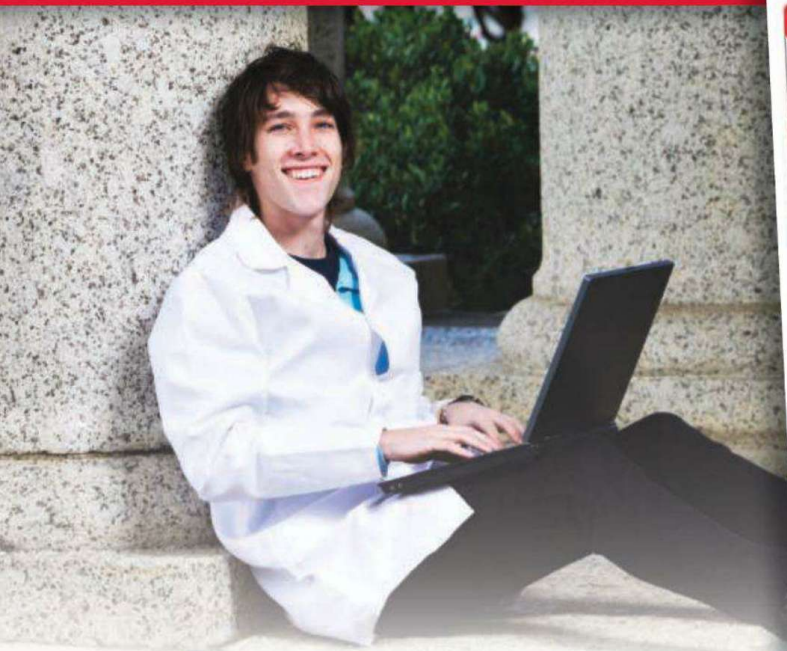
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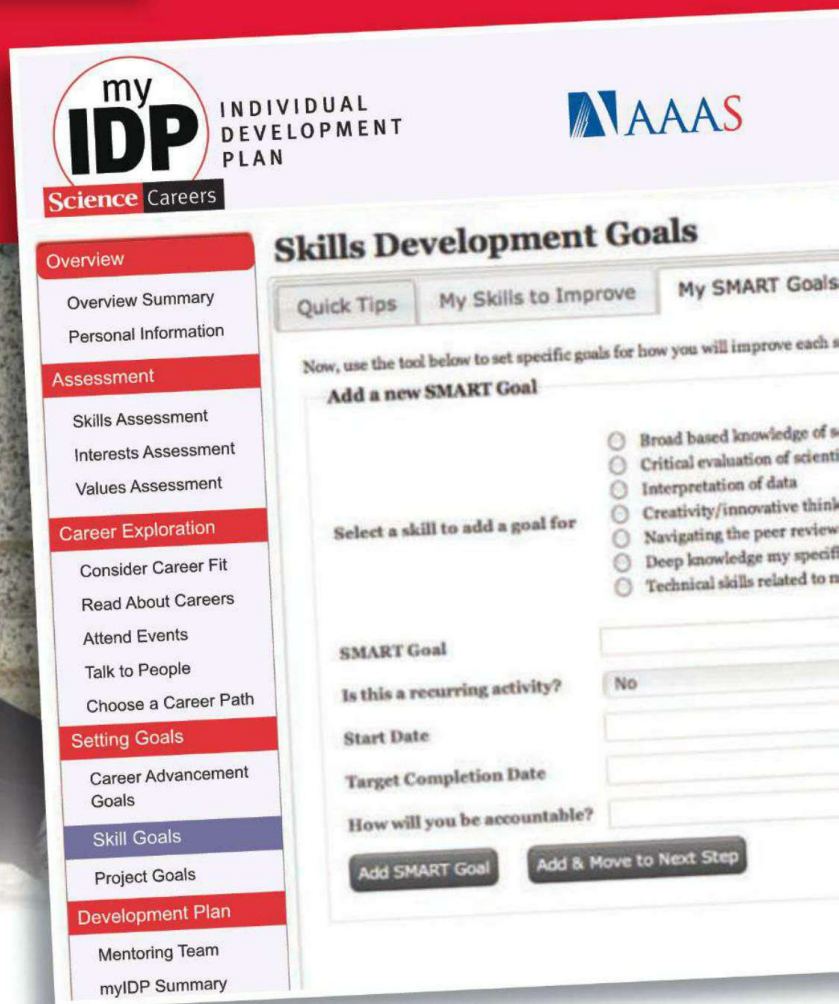
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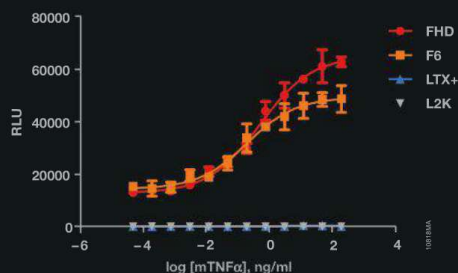
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